"COMPARATIVE EVALUATION OF SEALING ABILITY OF MINERAL TRIOXIDE AGGREGATE, BIODENTINE, ENDOSEQUENCE ROOT REPAIR MATERIAL AND BONE CEMENT AS RETROGRADE FILLING MATERIALS IN ULTRASONICALLY PREPARED ROOT ENDS USING CONFOCAL LASER SCANNING MICROSCOPE – AN *IN VITRO* STUDY"

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV – CONSERVATIVE DENTISTRY

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RAJAS DENTAL COLLEGE AND HOSPITAL

THIRURAJAPURAM, KAVALKINARU – 627 105, TIRUNELVELI

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CERTIFICATE BY THE GUIDE

This is to certify that this dissertation entitled **"Comparative evaluation of sealing ability of Mineral Trioxide Aggregate, Biodentine, Endosequence root repair material and Bone cement as retrograde filling materials in ultrasonically prepared root ends using confocal laser scanning microscope - an in vitro study"** is a bonafide research work done by **DR.G.PRIYA JOHNSON** under my guidance during her postgraduate study period between **2013 - 2016.**

This Dissertation is submitted to **THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY** in **CONSERVATIVE DENTISTRY AND ENDODONTICS – BRANCH IV.** It has not been submitted partially or fully for the award of any other degree or diploma.

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This is to certify that the dissertation entitled **"Comparative evaluation of sealing ability of Mineral Trioxide Aggregate, Biodentine, Endosequence root repair material and Bone cement as retrograde filling materials in ultrasonically prepared root ends using confocal laser scanning microscope - an *in vitro* study"**, is a bonafide research work done by **DR.G.PRIYA JOHNSON** under the guidance of **DR. R. JONATHAN EMIL SAM, M.D.S.**, Professor and Head**,** Department of Conservative Dentistry and Endodontics, Rajas Dental College and Hospital, Kavalkinaru, Tirunelveli-627105.

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LIST OF ABBREVIATIONS USED

(IN ALPHABETICAL ORDER)

ABBREVIATION	WORD EXPLANATION
ANOVA	Analysis Of Variance
BA	Bioaggregate
CEJ	Cemento Enamel Junction
CFU	Colony Forming Units
CLSM	Confocal Laser Scanning Microscope
CPC	Calcium Phosphate Cement
DC	Diamond Coated
EBA	Ethoxy Benzoic Acid
E. Faecalis	*Enterococcus faecalis*
EDTA	Ethylene Diamine Tetra Acetic Acid
ERRM	Endosequence Root Repair Material
Er:YAG	Erbium:Yttrium Aluminium Garnet
FT-IR	Fourier Transform Infrared Spectroscopy
GIC	Glass Ionomer Cement
IRM	Intermediate Restorative Material

LSM	Linux Software Map
MTA	Mineral Trioxide Aggregate
Ni Ti	Nickel Titanium
p Value	Probability Value
PBS	Phosphate Buffered Saline
PMMA	Polmethylmethacrylate
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SPSS	Statistical Package for Social Sciences
SS	Stainless Steel
US	Ultrasonics
WMTA	White Mineral Trioxide Aggregate
XRD	X-Ray Energy Dispersive Analysis
ZOE	Zinc Oxide Eugenol

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INTRODUCTION

Endodontics is defined as "the branch of dentistry that is concerned with the morphology, physiology and pathology of the human dental pulp and the periradicular tissues". Any pathology which involves only the pulp can be successfully treated by conventional or non surgical root canal therapy with a success rate of 96%[1.] But if the pathology extends to the periradicular tissues, chances of success is comparatively lower and may require surgical intervention for establishing complete debridement and hermetic seal between the intraradicular and extraradicular systems[2].

The major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth. Biofilms are sessile microbial communities composed of cells irreversibly attached to a substratum and interface or to each other[3]. Cells located more deeply in the biofilm are exposed to environmental conditions that differ from those at the surface including decreased oxygen tension. This results in altered phenotypes in terms of growth rate and gene transcription that might facilitate certain virulence characteristics[4].

The key pathogens in post treatment endodontic infections are *Enterococcus faecalis (E.faecalis), Pseudomonas aeruginosa, Staphylococcus species, Eschericia coli* and *Candida species. E.faecalis* is one of the species that can best adapt to and can tolerate the ecologically demanding conditions in the filled root canal. Eradication of *E.faecalis* from the root canal with chemo-mechanical techniques are difficult. Even following complete preparation and obturation, if the resultant microbial ecosystem is amenable to bacterial survival, a lesion may not heal and the pathology persists[5].

Percolation of tissue fluids could provide nutrients for the residual microorganisms to proliferate and reach sufficient numbers to induce or perpetuate inflammation. Endodontic periapical surgery remains the lifebuoy of the tooth if non surgical retreatment is unsuccessful due to persistent infection or if it is not feasible for adequate disinfection due to anatomic complexities. It has also been noted that it is impractical to carry out conventional root canal retreatment as in cases of a large root canal post well cemented in an already weakened root or in cases of unsuccessful non-surgical retreatment. It is also performed as an adjunct in perforation repair or to remove extruded material in cases where root or tooth resection is required and when a biopsy is required for investigation and exploration[6].

The surgical protocol involves root end resection of apical 3mm, as it is the area where 98% of apical ramifications, accessory and lateral canals are present. It is then followed by root end preparation and sealing with retrograde obturation materials. Apparently elimination of the persistent microorganisms in the apical third and depriving them of nutrient supply by providing adequate seal is the mechanism behind endodontic surgery[7].

Traditionally, root-end preparation has been carried out with burs that have disadvantages such as limited access, risk of perforation of the root and creating a 45º bevel. A 45º buccolingual bevel could increase the apical leakage because the permeability of the dentinal tubules are increased by the bevel angle which exposes more number of dentinal tubules which increases the chances of microleakage. To overcome these drawbacks of burs, ultrasonic tips were advocated[8]. The advantages of this technique would be a minimal or no bevel after resection of the root-end, smaller cavities with more preservation of tooth material, a deeper preparation in the

root-end that is better directed into the root canal, and a better preparation of anatomical difficulties, such as an isthmus[8].

The quality of the apical seal is considered as a critical factor in the success of periradicular surgery. The main objective of a root end filling material is to provide an apical seal that prevents the movement of bacteria and diffusion of bacterial products from root canal system into periapical tissues i.e microleakage which is the most common cause for endodontic failure. It should also be biocompatible, insoluble, easy to manipulate, radiopaque, insensitive to moisture, adherent to dentin, bacteriocidal or bacteriostatic and promote cementogenesis[9].

Various root end filling materials which have been widely used in the past were amalgam, glass ionomer cement, zinc oxide eugenol cement, gold foil, Intermediate restorative material etc..,. But recently many new alternative retrograde filling materials have evolved like Mineral Trioxide Aggregate (MTA), Biodentine, Polymethylmethacrylate bone cement and Endosequence root repair material which promises to provide a better hermetic seal.

MTA is a tricalcium silicate based cement used for retrograde filling owing to its biocompatibility, ability to set in the presence of moisture, ability of hard tissue induction and superior sealing ability[10].

Biodentine is tricalcium silicate based cement which is an “in-house synthesized” tricalcium silicate which guarantees high purity. It is used for retrograde filling as it has better consistency, better handling property, biocompatibility, radiopacity, ability to stimulate reactionary dentin and excellent sealing property[11].

Bone cement is a polymethylmethacrylate based cement which has been applied in dentistry as a retrograde filling material, as it has good handling properties, faster setting time, ability to set in moist environment and has a good marginal adaptation[12].

Endosequence root repair material, a bioceramic material is based on calcium silicates used for retrograde filling as it is biocompatible, hydrophilic, antibacterial, has a high pH, excellent radiopacity and sealing ability[13].

Sealing ability which is the prime requisite feature of retrograde filling materials refers to a material's ability to resist microleakage through the entire thickness of material[14]. For evaluation of sealing ability, various methods have been used like dye penetration, fluid filtration, electrochemical methods and bacterial leakage[15,16]. But three dimensional techniques reveal more relevant patterns than conventional sectioning technique that only provides two dimensional view.

Confocal laser scanning microscopy (CLSM) is a non destructive tool for viewing the subsurface tomography of hard tissues. It provides three dimensional images by means of microscopic tomography. It does not need demineralisation and specific sectioning technique of specimens. So the sample is free of artifacts induced by drying, sectioning or other pretreatments that are required by other analytical techniques[17].

So this study was aimed to evaluate the sealing ability of MTA, Biodentine, Bone cement and ERRM as retrograde filling in ultrasonically prepared root end cavities using CLSM.

AIMS AND OBJECTIVES

AIM

To evaluate the sealing ability of Mineral trioxide aggregate, Biodentine, Bone cement and Endosequence root repair material as retrograde filling materials in ultrasonically prepared root ends.

OBJECTIVE

To evaluate the sealing ability of four root end filling materials on root ends prepared using ultrasonics by confocal laser scanning microscope.

NULL HYPOTHESIS:

There exists no difference between the sealing ability of the root end filling materials used in this study.

REVIEW OF LITERATURE

High and Russell[18] conducted a series of laboratory-based tests in 1989 to assess the suitability of three antibiotic containing cements such as Cyanomethacrylate-1G, Cyanomethacrylate-3G and Palacos R with gentamicin and compared them with Amalgam as retrograde filling materials. For dye diffusion tests, extracted incisor teeth were resected and retrograde root filled with one of the four test materials. They were then placed and sealed into a base of plastic tubing, by being dipped into molten Metro modelling wax and then held vertically with the root apex uppermost and 1 percent methylene-blue dye was introduced into the tubing under standardized conditions, so that a column 80 cm high and 0.5 cm diameter applied the same hydrostatic pressure over the tooth apex. At the end of the test period of forty eight hours, the tooth was sectioned using minimal water cooling, to avoid washing out of the dye, and dye penetration was assessed by measuring the distance to the point of maximum dye penetration along dentinal tubules from the filling material margin. Five teeth were tested for each material and dye penetration was measured from the sectioned tooth on a microscope stage. It was found that marginal leakage was much reduced and only evident microscopically. Though they significantly leaked more than Amalgam, Palacos Bone cement was found to give a satisfactory marginal seal to be ideally used as a root end filling material.

M.Torabinejad *et al*[19] conducted an *in vitro* study in 1993 to evaluate the sealing ability of Amalgam, super EBA and MTA as root end filling materials using Rhodamine B fluorescent dye and CLSM. Thirty single canal teeth were cleaned, shaped, and obturated with gutta percha and grossman sealer. After resection of root at apical 3mm, root end preparation was done of 3mm depth using a round bur in high speed handpiece. The roots were randomly divided into three groups and the root end

preparations were filled with the experimental materials. All roots were exposed to an aqueous solution of Rhodamine B flourescent dye for 24 hours, longitudinally sectioned, and the extent of dye penetration was measured using CLSM. Statistical analysis showed that MTA leaked significantly less than Amalgam and super EBA.

Holt and Dumsha[20] conducted an *in vitro* leakage study in 2000 to compare the root-end sealing ability of Amalgam with cavity varnish, composite with dentin bonding agent, and Super-EBA with Bone cement. Eighty single-rooted teeth were instrumented and obturated with gutta percha, resected and retro prepared. The teeth were then randomly divided into 4 groups of 20, with each group receiving one of the previously mentioned retrofill materials. The Bone cement group was either etched or unetched. The teeth were immersed in silver nitrate and developer for leakage assessment. The teeth were grooved and split longitudinally to measure leakage. Statistical analysis showed that Amalgam leaked significantly less than Super-EBA and unetched Bone cement; composite leaked significantly less than Super-EBA. Amalgam was not statistically different from composite or etched Bone cement. No significant difference between Composite and both Bone cements was noted, nor between both Bone cements and Super-EBA. They concluded that Bone cement appears to seal comparably with composite and Super-EBA.

Aqrabawi[21] conducted a randomised control trial in 2000 to compare the apical microleakage of MTA following retrograde root filling with Amalgam and EBA. The root canals of 79 extracted teeth were instrumented and obturated with vertically condensed gutta-percha. Each tooth was apically resected and the apex was prepared ultrasonically to 3 mm depth and the root surface isolated with nail varnish. Teeth were divided randomly into three groups of 25 teeth each. First group was

retrofilled with Amalgam, second group with EBA and the third group with MTA. Following immersion in 1% methylene blue dye for 72 hours, the roots were sectioned and the depth of dye penetration was evaluated by a stereomicroscope at 10x magnification. 56% of the group filled with Amalgam and 20% of the group filled with EBA showed dye leakage beyond the retrofilling material whereas the MTA group showed none. The chi-squared test revealed a statistically significant difference among all three groups ($p < 0.05$). They concluded that MTA cement provides a better seal than Amalgam and EBA cement when used as retrograde filling material.

W. A. De Almeida *et al*[22] conducted an *in vitro* study in 2000 to evaluate the apical sealing ability of three endodontic sealers. The root canals of 99 extracted human maxillary central incisors were prepared sequentially 2 mm beyond the apical foramen with a size 55 Nitiflex file. The teeth were divided into three experimental groups and obturated with cold gutta-percha by lateral condensation with one of the following sealers: group 1, zinc oxide and eugenol sealer (Fill Canal); group 2, glass ionomer sealer (Ketac-Endo) and group 3, epoxy resin sealer (AH Plus). The teeth were covered with nail varnish to within 1 mm of the apical foramen and immersed in 2% methylene blue in a reduced pressure environment for 24 hr. After this period, the teeth were washed and cut longitudinally for apical leakage analysis. The values were obtained from the maximum depth of leakage as well as the average between the maximum and minimum values observed for each group. Statistical evaluation of the results showed no significant difference in the leakage between zinc oxide eugenol sealer and glass ionomer sealer ($p > 0.05$). Leakage with AH Plus was significantly less ($p < 0.01$) than with the other sealers.

Evans *et al*[23] conducted a study in 2000 to measure the accuracy of three dimensional imaging of small mammalian teeth using CLSM. Moulding and casting of the teeth were first performed, followed by confocal fluorescence imaging. Accuracy and precision of the scanned structures were tested in morphometric studies by using a new technique to measure the noise in the scan of a three-dimensional surface. The linear and angular dimensions of the scans were compared with measurements made using traditional morphological tools. It is shown that measurements can be taken with less than 4% difference from the original object. Teeth of the bat species *Chalinolobus gouldii* were scanned and measured to show the potential of the techniques. Methods for visualizing the small teeth in three-dimensional space, and animating the teeth in occlusion, show the power of this approach in aiding a three-dimensional understanding of the structure and function of teeth and other three-dimensional structures.

Peters CI *et al*[24] conducted an *in vitro* study in 2001 to compare the appearance of root-end cavity preparations and the time required to prepare them using prototype US diamond-coated (DC) and SS retrotips. In twenty four molar teeth, 48 root-end cavities were prepared ultrasonically in the palatal, mesio-buccal, distal and mesial root-ends using DC and SS retrotips, alternately. Replicas of the resected root tips and the root-end cavities were examined under SEM, evaluating (i) incidence and extent of dentine cracks (ii) minimum remaining thickness of the dentine walls and (iii) surface quality of the resected root ends. The time taken to complete the preparation was also recorded. Means of these parameters were compared for both types of retrotips using non parametric tests. They found that after preparation one root-end cavity shaped by an SS retrotip had a microcrack visible at 23x magnification. Four and seven other root-ends had crazed surfaces in the DC and

SS groups, respectively ($p > 0.05$). Remaining minimum dentine thickness was 0.56 ± 0.28 mm and 0.71 ± 0.24 for the DC and SS groups, respectively, and this difference was significant ($p < 0.05$). A root-end cavity in one specimen in the DC group was perforated. Preparation times ranged from 25 sec to 36 sec and were significantly lower for DC tips ($p < 0.01$) than the SS tips. The time required to prepare root-end cavities also differed between roots; root-end preparation in mandibular molars was more time consuming. They concluded that a better quality surface was produced by the prototype DC retrotips, in less time than the SS retrotips, which in turn caused fewer cracks than previously reported. DC retrotips removed more dentine than SS retrotips and should therefore be used with care to avoid overpreparation or perforation.

Maddalone.M *et al*[25] conducted an *in vitro* study in 2003 to monitor the outcome of periradicular surgery in a group of teeth treated with microsurgical technology and ultrasonic root-end preparation. Surgical procedure was done in one hundred and twenty eight teeth with failed conventional root canal treatment. The surgical procedure was completed using ultrasonic retrotips. A zinc ethoxy benzoic acid (EBA) reinforced material was used to seal the root-end cavities. Lesions were examined radiologically at 1, 3, 6, 12, 24 and 36 month intervals. Radiographs were independently analysed according to a previously published classification. Of the 120 teeth examined, the overall success rate was 92.5%; 94 healed with complete bone healing of the surgical cavity, 17 were considered to have healed by apical scar formation, 4 demonstrated uncertain healing and 5 were considered failures. Eighty of 120 teeth examined had successfully healed from a radiological point of view within 12 months. They concluded that modern surgical endodontic procedures associated and EBA root end fillings were successful over 3 years in 92.5% of cases.

Pinheiro *et al*[26] conducted an *in vitro* study in 2003 to identify the microbial flora within root canals of teeth with failed root canal treatment and to determine the association of the various species with clinical features. Sixty root filled teeth with persisting periapical lesions were selected for this study. During nonsurgical endodontic re-treatment, the root-filling material was removed and the canals were sampled. Microbial sampling, isolation and species determination were performed using advanced microbiological techniques for anaerobic species. The association of microbiological findings with clinical features was investigated. They found that of the microbial species isolated, 57.4% were facultative anaerobic species and 83.3% Gram-positive microorganisms. *E.faecalis* was the most frequently recovered bacterial species. Obligate anaerobes accounted for 42.6% of the species and the most frequently isolated genera was *Peptostreptococcus,* which was associated with clinical symptoms ($p<0.01$). Significant associations were also observed between: (a) pain or history of pain and polymicrobial infections or anaerobes ($p<0.05$); (b) tenderness to percussion and *Prevotella intermedia / P. nigrescens* ($p<0.05$); (c) sinus and *Streptococcus species* ($p<0.001$) or *Actinomyces species* ($p<0.01$); (d) coronally unsealed teeth and *Streptococcus species* or *Candida species* (both with $p<0.01$). They concluded that the microbial flora in canals after failure of root canal treatment were limited to a small number of predominantly Gram-positive microbial species. Facultative anaerobes, especially *E. faecalis,* were the most commonly isolated microorganisms, however, polymicrobial infections and obligate anaerobes were frequently found in canals of symptomatic root canal treated teeth.

B.S.Chong *et al*[27] conducted a clinical study in 2003 to assess the success rate of MTA as a root end filling material. A standardised surgical technique was

employed in which root end was resected perpendicularly and root end prepared ultrasonically and filled. Radiographic comparison was done at 12 and 24 months. From the results obtained from 122 patients after 12 months review period and from 108 patients after 24 months review period, it was found that highest number of teeth with complete healing was observed when MTA was used as a root end filling material. They concluded that use of MTA as a root end filling material resulted in high success rate.

E. G. Kontakiotis *et al*[28] conducted an *in vitro* study in 2004 to determine the impact of root-end resection and root-end cavity preparation on leakage of different tooth groups. The root canals of 48 roots which includes 16 mandibular premolars (G1), 16 mandibular incisors (G2) and 16 maxillary incisors (G3) which were of uniform length of 12 mm were enlarged using a modified 'balanced force' technique and filled with gutta-percha and sealer using lateral compaction. After setting, leakage along the canal was measured using a fluid transport model. Root-end resection and root-end cavity preparation were then performed, leaving roots 10 mm in length with root fillings of 7 mm. Fluid transport was measured again along the remaining root fillings of all groups using the same experimental conditions. Results of leakage before and after root-end resection were analysed. A total of 31% of the roots leaked before and 54% after root-end preparation. Root end resection and root-end cavity preparation compromised the seal of 7 mm root fillings in all tooth groups. Maximum leakage was found with mandibular incisors followed by mandibular premolars and least microleakage was found with maxillary incisors.

Georgia E. Nikoloudaki *et al*[29] conducted an *in vitro* study in 2004 to compare the sealing ability and marginal adaptation of four restorative materials

namely MTA, Biodentine, portland cement, and resin modified GIC when used as repair materials in iatrogenic furcation perforations. Eighty-four molars were treated endodontically, perforated in the middle of the pulp chamber floor with a round bur and separated randomly into 4 groups of 20 teeth each, while 4 teeth were used as positive and negative controls. The teeth were embedded in a moistened sponge and the perforations were filled with the appropriate restorative materials: Group 1: Biodentine; Group 2: MTA Angelus; Group 3: GC Fuji lining LC Paste Pak; Group 4: Aquafix Portland cement. The teeth remained in the soaked sponge for 28 days and then were submerged in basic fuchine solution 1% for 48 hours. Dye penetration was evaluated after longitudinal sectioning of the teeth. Statistical analysis revealed that perforations restored with MTA exhibited the least microleakage with statistical significant difference among the other three groups ($p < 0.05$). The worst sealing ability was observed in the teeth restored with Aquafix Portland cement. No statistical significant difference was found between the groups of Biodentine and FC Fuji Lining Paste ($p = 0.066$).

lntekhab Islam[30] conducted an *in vitro* study in 2005 to compare the sealing ability of ProRoot MTA, ordinary Portland cement and white Portland cement as root-end filling materials. Twenty-four single-rooted human premolars were prepared and obturated using standard techniques, then retrofilled with the test materials. The prepared teeth were immersed in 1% methylene blue dye for 72 hours and then assessed for dye leakage. The depth of dye penetration was measured and expressed as a percentage of the length of the retrofilling. Data was analysed using ANOVA and Fisher's least significant test ($p < 0.05$). He found that none of the teeth in any of the test groups showed leakage beyond the retrofillings.

K.Kamburoglu *et al*[31] conducted an *in vitro* study in 2007 to compare measurements obtained by micro-CT with those obtained by CLSM in simulative internal resorption cavities. An extracted human maxillary central incisor was divided into two in the coronal plane. Four artificial internal resorption cavities were prepared with standardised burs on each section, and diameters and volumes were measured using a CLSM and a desktop cone beam micro-CT-40. Differences between quantitative measurements for both methods were tested and they concluded that micro-CT significantly underestimated the diameters and volumes when compared to CLSM.

Jan De Lange *et al*[32] conducted an in vivo study in 2007 to evaluate the potential benefit of an ultrasonic device in apical surgery on the outcome of treatment. 399 patients were referred from surgery department with a previous history of RCT. These root canal treated tooth were selected and divided into two groups for randomisation. In one group the preparation of the root end cavity was carried out by a bur and in the other group by the use of an US device. Periapical surgery was done and in all cases the root end fillings were made of IRM. A radiograph of the treated tooth was made immediately postoperative and subsequent intervals of 6 months and 1 year after treatment. Clinical examination was performed later at 6 months and 1 year after therapy. A final radiographic analysis of the treatment outcome was performed by 2 surgeons who were blinded for the therapy. The outcome was very clearly in favour of the ultrasonic device. He concluded that the use of an ultrasonic device in apical surgery showed a clear benefit over the traditional treatment.

D. Saini *et al*[33] conducted a study in 2008 to compare the microleakage of three root end filling materials MTA, GIC and Silver GIC (Miracle

Mix) using dye penetration technique under stereomicroscope. Forty-five extracted human maxillary central incisors were instrumented and obturated with gutta percha using lateral compaction technique. After one week, teeth were apically resected at an angle of 90°to the long axis of the root and root end cavities were prepared. The teeth were divided into three groups of fifteen specimens each and were filled with MTA, GIC and miracle mix. The samples were coated with varnish and after drying, they were immersed in 1% methylene blue dye for 72 hours. The teeth were then rinsed, sectioned longitudinally and observed under stereomicroscope. The depth of dye penetration was measured in millimeters. They concluded that MTA is a better material as root end filling material to prevent microleakage, in comparison to GIC and Miracle Mix.

El Aasser M *et al*[34] conducted an *in vitro* study in 2009 to compare the sealing ability of three different root-end filling materials, namely: MTA, composite resin with two-step self etching adhesive and composite resin with two-step adhesive with phosphoric acid etching in root-end filling cavities prepared by burs or US retrotips. The roots were resected in different angulations like 45º beveled and non-beveled roots. 120 single canal teeth were divided into two groups according to beveling of resected root (45º bevel and no bevel). The two groups were further divided into 4 subgroups according to instrument used in root-end cavity preparation (types of burs and US tips). The subgroups were divided into subdivision according to material used for filling. Dye penetration method was used to evaluate the sealing ability of the fillings. The results of the study concluded that the best sealing ability gained with no beveling of resected root, using of US tips in root-end cavity preparation and MTA as root-end filling material.

Akira Mitsuhashi *et al*[35] conducted a study in 2009 to compare MTA root fillings under three conditions - with unaided vision, with loupes, and with microscope. Cavities were prepared on eight extracted human teeth using laser. MTA was filled into the root cavity under the three working conditions. The marginal adaptation of each cavity was observed and evaluated under CLSM. The specimens were also subjected to dye penetration test to evaluate marginal leakage. They found that the CLSM evaluations revealed that the fillings performed under the operating microscope showed better marginal adaptation and the infiltration of methylene blue dye decreased with the fillings performed under microscope. They concluded that the procedures performed under operating microscope provided better results than those obtained with unaided vision or with dental loupes.

Letecia Kirst *et al*[36] conducted an *in vitro* study in 2010 to evaluate the sealing ability of MTA and Amalgam in different root end preparations and resection bevel angles. Eighty extracted human single root teeth were cleaned, shaped and obturated with guttapercha and roots were divided into 8 groups. 40 teeth were resected at 45º angle and 40 teeth were resected at 90º angle. They are divided into 4 subgroups of 20 teeth each. Each subgroup was prepared using ultrasonic tip and carbide bur. They were further divided into 8 groups among which 4 groups were filled with MTA and 4 groups were filled with Amalgam. After retrofilling, specimens were immersed in 0.2% Rhodamine B dye. The teeth were then sectioned transversely into 3 sections, 1 mm far from each other. Sections were glued to millimeter-graph paper with ethyl cyanoacrylate and viewed under stereomicroscope. They concluded that the angle of apicoectomy and the type of root end preparation did not affect the degree of microleakage and MTA yields better results because it produced less leakage than Amalgam.

Amany E. Badr[12] conducted an *in vitro* study in 2010 to evaluate the marginal adaptation and cytotoxic effect of PMMA Bone cement, MTA and Amalgam as root end filling materials**.** Thirty extracted human single-rooted teeth were cleaned, shaped, and obturated with gutta-percha and AH 26 sealer. The roots tips were removed; root-end cavities were prepared and filled with the 3 tested materials (Bone cement, MTA, and Amalgam). Impressions of retrofilled root ends were taken by polysiloxane impression material, and positive replicas were fabricated by using epoxy resin. The original roots were longitudinally sectioned into 2 halves; both the replicas and longitudinal sections were prepared for SEM to measure the gaps at the material-dentin interface. Human periodontal ligament fibroblast tissue culture was used to assess the cytotoxicity of the 3 tested materials. The obtained data revealed that both Bone cement and MTA exhibited a better adaptation to the dentinal walls than that of Amalgam. Also, the cytotoxicity testing showed that Bone cement had a comparable cytotoxic effect on fibroblast cells with MTA; both root-end filling materials showed less cytotoxicity than that of Amalgam. They concluded that PMMA Bone cement could be considered as a promising root end filling material**.**

Clovis. M .Bramante *et al*[37] conducted an *in vitro* study in 2010 to evaluate the effect of sputter-coating for SEM analysis on the formation of cracks on root end surfaces after retrograde cavity preparation with US tips. Root end cavities were prepared with either DC retrotips or noncoated SS retrotips. Impressions were taken before and after retrograde cavity preparation. The resected root apices and their respective impressions were examined using SEM and the presence, extension and numbers of cracks were recorded after each procedure. They observed that the number of cracks observed directly on root end surfaces was larger than that observed in the impressions. They concluded that cracking was mostly produced by the sputter

coating process required for SEM analysis and the incidence of cracks was greater in impressions of cavities prepared with non coated SS tips than those that replicated cavities prepared with DC retro tips.

Juan-Ignacio Rosales-Leal *et al*[38] conducted an *in vitro* study in 2011 to evaluate the effect of cavity preparation with microburs and DC ultrasonic tips on the microleakage and marginal fit of six end-root filling materials namely Amalgam, ZOE, GIC, compomer, MTA and composite. Cavity preparation was performed with microburs or diamond US tips in single-rooted teeth. The seal was evaluated in two experiments: a microleakage assay on the passage of dye to the interface; and a SEM study and analysis of epoxy resin replicas, measuring the size of gaps in the interface between filling material and cavity walls. They concluded that Clearfil and MTA obtained a hermetic seal due to their excellent marginal fit and are the most recommended materials for clinical use, taking account of their sealing capacity. US cavity preparation is preferable because it improves the seal and marginal fit of materials.

Uma Nair *et al*[17] conducted an *in vitro* leakage study in 2011 using *E.faecalis* to compare the sealing ability of two root end filling materials namely ERRM and MTA. Forty single-rooted teeth were instrumented, obturated with gutta-percha, root-end resected, and retrofilled with two different materials: WMTA (n =15) and ERRM (n=15). Unfilled specimens (n=10) which received no retrofill were used as controls. All groups received *E.faecalis* in a created reservoir coronal to the root filling and the presence of microleakage was evaluated by counting the CFU from each specimen. The results were analyzed with One-way ANOVA. They found that there was no significant difference in leakage between the two experimental groups,

but there was significant difference with the control (p<05). They concluded that ERRM is equivalent in sealing ability to WMTA when used as root-end filling material.

Han & T. Okiji[39] conducted an *in vitro* study in 2011 to compare the Ca and Si uptake by root canal dentine in the presence of PBS from Biodentine and WMTA. Root canals of bovine incisor root segments were instrumented, filled with either Biodentine or MTA (n= 20 each) and then immersed in Ca-and Mg-free PBS for 1, 7, 30 or 90 days (n=5 each). Unfilled, unimmersed dentine specimens (n=5) served as controls. The specimens were sectioned longitudinally, and the ultrastructure of the dentine-material interface and the elemental composition/ distribution in the material-dentine interface were analysed using a wavelength-dispersive X-ray spectroscopy electron probe microanalyser with image observation function. Data were statistically analyzed using one-way ANOVA. They found that along the material–dentine interface, both materials formed a tag-like structure that was composed of either Ca and P rich crystalline deposits or the material itself. The width of a Ca and Si rich layer detected along the dentine layer of the material–dentine interface increases over time. The Ca and Si rich layer width was significantly larger (p< 0.05) in Biodentine than MTA at 30 and 90 days. They concluded that both Biodentine and MTA caused the uptake of Ca and Si in the adjacent root canal dentine in the presence of PBS. The dentine element uptake was more prominent for Biodentine than MTA.

Ordinola-Zapata *et al*[40] conducted an *in vitro* study in 2012 to evaluate the residual biovolume of live bacterial cells, the mean biofilm thickness and the substratum coverage found in biofilms by treating them with different endodontic irrigant solutions. Twenty-five bovine dentine specimens were infected intraorally

using a removable orthodontic device used as a splint. Five samples were used for each irrigant solution: 2% chlorhexidine, 1% sodium hypochlorite (NaOCl), 10% citric acid, 17% EDTA and distilled water. The solutions were used for 5 min. The samples were stained using the Live/Dead technique and evaluated using CLSM. Differences in the amount of total biovolume, number of surviving cells, mean biofilm thickness and substratum coverage of the treated biofilms were determined using non parametric statistical tests ($p< 0.05$). They found that similar values of biovolume total, biovolume of live subpopulations and substratum coverage were found in 2% chlorhexidine, 10% citric acid, 17% EDTA and distilled water-treated biofilms ($p> 0.05$). The lower values of the studied parameters were found in 1% NaOCl treated dentine ($p< 0.05$) with the exception of the mean biofilm height criteria that did not reveal significant differences among the irrigant solutions ($p> 0.05$). They concluded that 1% sodium hypochlorite was the only irrigant that had a significant effect on biofilm viability and architecture.

M. Matos Neto *et al*[41] conducted an *in vitro* study in 2012 to assess the effectiveness of three systems of root canal preparation to reduce *E.faecalis* within root canals. Twenty-four human single-rooted canine teeth were standardized to a length of 17 mm and the canal contents removed using a size 20 K-file, as the last apical file. After irrigation and sterilization, the canals were contaminated with *E. faecalis* and incubated for 21 days at 37ºC in a 5% Co_2 atmosphere. Then, the teeth were divided into three groups for mechanical preparation with: ProTaper rotary system, ProTaper manual system and manual K-files. Samples of the root canal contents, before and after the debridement, were collected with sterile paper points for 1 min. Then, the samples were diluted and plated in Brain Heart Infusion (BHI) agar. The colony forming units (CFU) were counted and the percentage reduction

calculated. The reduction and CFU/ml were compared between groups. They found that there was a significant reduction in the number of CFU/ml before and after debridement for all the systems used and all the three instrumentation systems reduced *E. faecalis* counts to a similar degree.

Noushin Shokouhinejad *et al*[42] conducted an *in vitro* study in 2012 to evaluate the bioactivity of Bioaggregate (BA), ERRM and WMTA. Sixty horizontal root sections with standardized canal spaces were divided randomly into 3 groups (n = 20) and filled with WMTA (groups 1 and 2), BA (groups 3 and 4) or ERRM putty (groups 5 and 6). The specimens of groups 1, 3 and 5 (n=10) were immersed in PBS for 1 week and those of groups 2, 4 and 6 (n=10) for 2 months. After the experimental periods, the specimens were processed for SEM observations. Precipitation of apatite crystals on the surfaces of the cements and/or at the dentine-cement interface was evaluated and analysed elementally by energy dispersive X-ray instrument. Analysis of specimens revealed various surface morphologies that were dependent on the material and immersion time in PBS. The formation of precipitates was observed on the surfaces of all materials at 1 week, which increased substantially over time. After 2 months, the surface of the cements was changed dramatically and consisted of a substantially greater amount of apatite aggregates. Interfacial layers in some areas of the dentine-cement interface were found only following 2 months of immersion. Precipitates on MTA revealed high peaks of Ca and Si after 1 week of immersion; after 2 months, high peaks of Ca and P were present. Precipitates on BA and ERRM displayed high Ca and P peaks after both 1 week and 2 months. They concluded that exposure of MTA, BA and ERRM to PBS resulted in precipitation of apatite crystalline structures that increased over time and hence it was proved that the tested materials were bioactive.

Daniel Torres-Lagares *et al*[43] conducted an *in vitro* study in 2012 to analyze the three-dimensional characteristics of the root-end cavity preparations completed in root apices of extracted teeth determining their area, perimeter, circularity and cavosurface angle, using CLSM. Thirty-two single rooted teeth were cleaned, shaped and obturated with gutta-percha and resin sealer. The root-end was resected at 3 mm of root apex using sterile water-cooled diamond blade. Apical root-end cavities were prepared ultrasonically under copious irrigation for 20 seconds. Four protocols were carried out, each one in eight teeth, as follows: cavities in groups 1 and 2 were prepared using stainless steel US tips (SST), and groups 3 and 4 were prepared using diamond-coated US tips (DCT). Two different intensities were used: maximum power (33 KHz) in groups 1 and 4, and medium power (30 KHz) in groups 2 and 3. Each root-end cavity was observed with a confocal microscope for the following data: root-end cavity area, root-end cavity perimeter, root-end cavity circularity, and cavo-surface angle of the root-end cavity. They concluded that confocal microscopy is a useful approach to study the three-dimensional characteristics of the root-end cavity.

M. Ahlquist *et al*[44] conducted a scanning electron microscope (SEM) study in 2013 to compare the cleanliness of the root canal walls following either a manual or a rotary technique of canal instrumentation. Manual filing using stainless steel files and powered instrumentation using ProFile rotary nickel–titanium files in a handpiece was performed on 10 extracted teeth. A solution of 0.5% sodium hypochlorite was used for irrigation. The roots were cut longitudinally and the canal walls were examined for debris and smear layer at the apical, middle and coronal level. Significantly less debris was found in the apical region using the manual filing technique ($p < 0.05$) and no significant differences could be found at middle and coronal levels. Overall,

significantly less debris was found on the root canal walls using the manual technique thereby producing more cleaner canals than Profile rotary technique

Han and Okiji[45] conducted an *in vitro* study in 2013 to compare the ability of white ProRoot MTA, Endosequence BC sealer and Biodentine to produce apatites and cause Ca and Si incorporation in adjacent human root canal dentine after immersion in Phosphate buffered saline (PBS). Root sections of human single rooted teeth were filled with one of the materials and immersed in PBS for 1, 7, 30 or 90 days (n=5 each). Morphology and elemental composition of surface precipitates and interfacial dentine were analysed using a wavelength-dispersive X-ray spectroscopy electron probe microanalyser with image observation function. Ca and Si incorporation depths in the interfacial dentine were measured. In addition, the amount of Ca ions released from the test materials was measured by EDTA titration. They found that all materials produced surface precipitates of acicular or lathe like morphology with Ca/P ratio of 1.6 : 2.0. Within dentinal tubules, the three materials formed tag like structures that were frequently composed of Ca and P rich and Si poor materials, suggesting intratubular precipitation. Ca and Si incorporation depths were in the order of Biodentine followed by WMTA followed by BC sealer, with a significant difference between BC sealer and the others at several time-points. The concentration of released Ca ions was in the order of Biodentine followed by WMTA followed by BC sealer with significant differences between the materials. They concluded that when compared with Biodentine and WMTA, BC sealer showed less Ca ion release and did not show Ca and Si incorporation in human root canal dentine when immersed in PBS for up to 90 days.

Seda Aydemir *et al*[46] conducted a study in 2013 to evaluate and compare the dentinal walls of root end cavities for the presence of cracks after cavity preparation using US retrotips and Er: YAG laser. Fifty single rooted teeth were prepared by Protaper NiTi rotary system and obturated by lateral condensation. Three milimeters of root-end was resected. Twenty teeth were prepared with US retrotip (Group 1), 20 teeth with Er: YAG laser (Group 2), and 10 teeth without retropreparation (control group). The root-end surfaces were examined under SEM. Then the cracks of the resected root surfaces were evaluated on microphotographs. They concluded that there was no significant difference as detected between US Group and Laser Group for complete, incomplete, intradentinal, and total number of cracks.

Trisha Charland *et al*[47] conducted an *in vitro* study in 2013 to compare the ability of MTA and ERRM to set in the presence of human blood and minimal essential media. A model was created using PMMA blocks each prepared with 10 standardized wells (2mm diameter and 3mm depth). Prepared ProRoot MTA and ERRM were each placed in 6 separate blocks. The samples were distributed among the 4 different media (blood, minimal essential media, blood and minimal essential media, and sterile saline as the control). Each block was submerged for 4, 5, 6, 8, 24, 36, and 48 hours in an incubator at 37ºC with 100% humidity. The results revealed that regardless of the type of media exposure, neither of the materials set at 4 or 6 hours. ERRM was not set at 48 hours, whereas all of the MTA samples were set at 36 hours. They concluded that it would be prudent to wait at least 36 hours for MTA to set and even longer to allow ERRM to set before continuing either endodontic or restorative procedures.

Hirschberg *et al*[48] conducted an *in vitro* study in 2013 to compare the sealing ability of MTA and ERRM using a bacterial leakage model. Root canals of 60 single rooted extracted teeth were prepared and obturated. Apical 3mm was sectioned at 90º to the long axis of root. Retro preparation was done using US surgical tip. Teeth were divided into 4 groups- group 1 filled with MTA, group 2 filled with ERRM, in group 3, gutta-percha without sealer was used as a positive control and group 4 was used as a negative control where the cavity was sealed with wax or nail varnish. Teeth were suspended in sterilised vials containing 3% phenol lactose and inoculated with *E. faecalis* through the occlusal openings. The samples were observed daily for leakage for 28 days. They found that 93% of ERRM samples leaked, compared to only 20% leakage in MTA group. They concluded that ERRM group exhibited more leakage than MTA group.

Sharad R.Kokate *et al*[49] conducted an *in vitro* study in 2013 to comparatively evaluate stereomicroscopically the microleakage of three root end filling materials MTA, GIC and Biodentine using dye penetration. Root end cavities were prepared in thirty extracted human maxillary central incisors. The teeth were then randomly divided into 3 groups of 10 specimens each & were filled with MTA, GIC and Biodentine and were immersed in 1% methylene blue dye for 72hrs. The teeth were then sectioned longitudinally and examined under stereomicroscope. The depth of dye penetration was measured in millimeters. They concluded that GIC, MTA and Biodentine exhibited microleakage with Biodentine showing the least microleakage of all.

Sabari Girish C *et al*[50] conducted an *in vitro* study in 2013 to compare the sealing ability of MTA, PMMA Bone cement and Chitra CPC when used as root end

filling material, using Rhodamine B dye evaluated under CLSM and to compare the seal of root ends prepared using an US retroprep tip and an Er: YAG laser using three different root end filling materials. Eighty sound, caries-free, mandibular premolars with single canals were shaped and obturated with gutta-percha and AH plus sealer. Root resections were done and the teeth were randomly allocated into two control groups of 16 teeth each and experimental groups of 48 teeth each. In half of the samples, the apical cavity was prepared using an US retro preparation diamond tip and in the rest of the samples, apical cavity preparation was performed using an Er: YAG hard tissue laser. They were further divided into five groups and the root end cavities were filled with MTA, PMMA Bone cement and Chitra CPC cement and one group was completely coated with nail varnish and one group was left without any filling. The roots were then totally immersed in a solution of Rhodamine B fluorescent dye for 24 hours. Using a diamond disc, each root was longitudinally sectioned into two halves. Each specimen was then examined as to the adaptation of the root end filling material to the cavity walls, the presence and absence of gaps and voids and the extent of dye penetration using CLSM. They concluded that PMMA Bone cement is a better material as root end filling material to prevent microleakage. MTA showed minimum microleakage. The difference between laser group and US group was found to be not statistically significant.

Grech *et al*[51] assessed in 2013 the composition of materials and products of a prototype cement of tricalcium silicate and radiopacifier and two commercially available tricalcium silicate cements, one of which was Biodentine. Their main purpose was to assess the effect of the additives used in commercial brands. The authors characterized the hydrated cements using SEM, X-ray energy dispersive analysis (XRD), X-ray diffraction, and Fourier transform infrared spectroscopy (FT-

IR). They concluded that Biodentine resulted in the formation of calcium silicate hydrate and calcium and hydroxide which leached in solution. The materials, when hydrated, consisted of a cementitious phase, rich in calcium, silicone, and a radiopacifying material. Biodentine was further described as having calcium carbonate in powder and the carbonate phase of the material was verified by XRD and FT-IR analysis. The Biodentine powder also had inclusions of calcium carbonate which were relatively large compared to cement particles. There were hydration products around the circumference of the calcium carbonate particles. The authors added that calcium carbonate acts as a nucleation site, enhancing the microstructure. Thus Biodentinee was proved to be bioactive.

Eppala Jeevani *et al*[52] conducted an *in vitro* study in 2014 to evaluate the sealing ability of Micro-Mega MTA, ERRM, Biodentine as furcation repair materials using a dye extraction leakage method. They grouped forty mandibular molars according to the material used. All the samples were subjected to orthograde and retrograde methylene blue dye challenge followed by dye extraction with 65% nitric acid. The samples were then analysed using ultraviolet visible spectrophotometer. It was found that Biodentine showed highest dye absorbance whereas ERRM showed lowest dye absorbance. They concluded that ERRM showed better sealing ability when compared with other root repair materials.

Betul Gunes *et al*[53] conducted an *in vitro* study in 2014 to evaluate the effects of different US surgical tips and power settings on microleakage of root end filling material. Root end preparation was done in 100 single rooted teeth which were divided into six groups. Diamond coated US surgical tip, zirconium nitride coated surgical tip and stainless steel (SS) tips were used for root end cavity preparation at

half power and high power settings. Evaluation of leakage was done using glucose penetration method. They concluded that root end filling showed the best sealing ability when the root end cavities were prepared with diamond coated US surgical tips at high power setting. The required cavity preparation time was also shortest with diamond coated retro tip at high power setting.

Neha Sharma *et al*[54] conducted an *in vitro* study in 2014 to evaluate the marginal adaptation of PMMA Bone cement, MTA, and Amalgam as root-end filling materials. Thirty extracted human single-rooted teeth were cleaned, shaped, and obturated with gutta-percha and AH 26 sealer. The roots tips were removed; root-end cavities were prepared and filled with the three tested materials (bone cement, MTA, and Amalgam). The original roots were longitudinally sectioned into 2 halves and longitudinal sections were studied under SEM to measure the gaps at the material/dentin interface. Half of the sections were observed for depth of dye penetration under stereomicroscope. For the longitudinal section specimens, SEM examination of the interface showed that Bone cement group had the least gap measurements (1.855 ± 0.380 μm), whereas maximum gaps were found in samples filled with Amalgam (4.48 ± 0.396 μm). All the retrograde root fillings showed leakage with dye penetration test. Although none had a score of zero, the scores were less in MTA and Bone cement than those for Amalgam. They concluded that both Bone cement and MTA exhibited a better adaptation to the dentinal walls than Amalgam.

Nowicka *et al*[55] conducted an *in vitro* study in 2014 on tomographic evaluations of reparative dentin bridge formation after direct pulp capping with calcium hydroxide, MTA, Biodentine and Single Bond Universal in human teeth.

Forty-four caries-free, intact, human third molars scheduled for extraction were subjected to mechanical pulp exposure and assigned to 1 of 4 experimental groups depending on the pulp capping agent used: calcium hydroxide, MTA, Biodentine, or Single Bond Universal. After 6 weeks, the teeth were extracted and processed for cone-beam computed tomographic imaging and histologic examination. Tomographic data, including the density and volume of formed reparative dentin bridges, were evaluated using a scoring system. The reparative dentin formed in the calcium hydroxide, MTA, and Biodentine groups was significantly superior to that formed in the Single Bond Universal group in terms of thickness and volume. The dentine bridges in the Biodentine group showed the highest average and maximum volumes. The mean density of dentine bridges was the highest in the MTA group and the lowest in the Single Bond Universal group. They concluded that the volume of reparative dentine bridges formed after direct pulp capping is dependent on the material used. Biodentine and MTA resulted in the formation of bridges with a significantly higher average volume compared with Single Bond Universal.

Pragna Mandava *et al*[56] conducted a study in 2015 to evaluate the apical microleakage of root end cavities filled with MTA, Biodentine and light cure GIC using two different cavity preparation techniques such as conventional bur preparation and ultrasonic tip preparation. Roots of eighty extracted single rooted human teeth (except mandibular incisors) with one canal, fully developed apices and without any major carious lesion are instrumented upto master apical file 40 K size and obturated with gutta percha and AH plus as sealer using lateral condensation technique. The teeth were then resected apically at 90° angle axis to the long axis of the root removing 3 mm of the apex. The teeth were divided in to four groups of 20 each and were restored with MTA, Biodentine and light activated GIC. One group

received no filling material. Each group is divided into two subgroups (a, b) of ten teeth each. In subgroup 1, retro preparation was done with US retrotip and in subgroup 2, retro preparation was done with conventional bur. The teeth were then immersed in 0.5% Rhodamine B dye for 48 hours. The teeth were split longitudinally and the interface between the restored material and the canal wall was observed under CLSM. Depth of dye penetration was examined under stereomicroscope. They concluded that MTA showed significantly less microleakage when compared to Biodentine and light cure GIC and there was no statistical difference between the ultrasonic retrotip preparation and conventional bur preparation.

Nicole Shinbori *et al*[57] conducted a retrospective study in 2015 to evaluate the clinical and radiographic outcome of root end surgery using ERRM as retrofilling material. Clinical records and periapical radiographs were collected from patients who have undergone endodontic microsurgery between 2009-2013 done by a single endodontist with ERRM as retrofilling material. On analysing the outcome based on healing they found that the overall success rate was 92%. They concluded that ERRM was a suitable and successful retrofilling material.

Ian Chen *et al*[58] conducted an animal study in 2015 to compare healing after root end surgery by using gray MTA and ERRM as root end filling material in an animal model where apical periodontitis was induced in 55 mandibular premolars of 4 healthy beagle dogs. After 6 weeks, microsurgical root end surgeries were performed. MTA and ERRM were used as root end filling materials. After 6 months, when healing of the periapical area were assessed using periapical radiographs, cone beam computed tomography and microcomputed tomography, it was found that ERRM was a biocompatible material similar to MTA with good sealing ability.

Shishir Singh *et al*[59] conducted an *in vitro* study in 2015 to compare solubility of Biodentinee with three commonly used root-end filling materials viz. GIC, IRM, and MTA. Twenty stainless steel ring moulds were filled with cements corresponding to four groups (n = 5). The weight of 20 dried glass bottles was recorded. Samples were transferred to bottles containing 5 ml of distilled water and stored for 24 hours. The bottles were dried at 105°C and weighed. This procedure was repeated for 3, 10, 30, and 60 days. Data was analyzed with one-way ANOVA. They found that Biodentine demonstrated significantly higher solubility than MTA for 30 and 60 day immersion periods. Statistical difference was noted between the solubility values of Biodentine samples amongst each of the five time intervals. They concluded that Biodentine exhibited higher solubility in comparison with all other cements.

Ankita Khandelwal *et al*[60] conducted an *in vitro* study in 2015 to compare sealing ability of MTA and Biodentine as root end filling material and also to compare the effect of different retro preparation techniques i.e. conventional bur v/s US tips on sealing ability of both the root end filling materials. 40 extracted human single rooted teeth were decoronated, and root canal treatment was performed. Teeth were stored in saline for 1 week. Following which, root ends were apically resected at 90° angle to a long axis of the root and prepared. The samples were randomly divided into two groups of 20 specimens each. Group I: MTA, Group II: Biodentine as the root end filling. Each group was subdivided as A (round bur preparation) and B (US tip root end preparation). Samples were stored in saline for 48 hours and then immersed in 0.5% Rhodamine B dye for 24 hours, sectioned and evaluated for leakage under CLSM. The results showed that Biodentine after US preparations showed significantly less microleakage than MTA after bur preparations and

concluded that Biodentine can be used as a replacement for MTA, as a root end filling material and US preparations facilitated good sealing ability.

Salin Nanjappa *et al*[61] conducted an *in vitro* study in 2015 to compare the sealing ability of MTA, Biodentine, and Chitra-CPC when used as root-end filling, evaluated under CLSM using Rhodamine B dye. To evaluate effect of US retroprep tip and an Er:YAG laser on the integrity of three different root-end filling materials. The root canals of 80 extracted teeth were instrumented and obturated with gutta-percha. The apical 3 mm of each tooth was resected and 3 mm root-end preparation was made using US tip (n= 30) and Er:YAG laser (n= 30). MTA, Biodentine, and Chitra-CPC were used to restore 10 teeth each. The samples were coated with varnish and after drying, they were immersed in Rhodamine B dye for 24 hours. The teeth were then rinsed, sectioned longitudinally, and observed under CLSM. Data were analyzed using one-way ANOVA and a post-hoc Tukey's test (R software version 3.1.0). Comparison of microleakage showed maximum peak value of 0.45 mm for Biodentine, 0.85 mm for MTA, and 1.05 mm for Chitra-CPC. They concluded that root-end cavities prepared with Er:YAG laser and restored with Biodentine showed superior sealing ability compared to those prepared with ultrasonics.

MATERIALS AND METHODS

S. no	Materials used	Brandname / Manufacturer details
1	Human maxillary central incisors	n=80
2	Endodontic access bur	Dentsply Maillefer,Ballaigues, Switzerland
3	Gates glidden drills No. 1 to 3	Mani, Japan
4	ISO size 10 to 80 K-Files	Mani, Japan
5	RC Prep EDTA conditioner	Premier dental products, North America
6	17% EDTA	De smear, Anabond Stedman Pharma,India.
7	5.2% sodium hypochlorite	Vensons India Pvt Ltd.,Delhi,India.
8	Normal saline	Nice chemicals Pvt Ltd.,Delhi,India.
9	Paper points	Hygienic, Coltene, USA
10	Lentulospiral	Dentsply Maillefer,Ballaigues, Switzerland
11	AH Plus Resin sealer	Dentsply, Newyork,USA
12	Guttapercha points size 15 to 50	Diadent International, Canada
13	Light cure Composite	G-aenial, GC Corp, Tokyo,Japan
14	Diamond disc	Axis dental, Kavo Kerr, Germany
15	Graduated periodontal probe	Hu-friedy, USA
16	MTA	Pro root MTA, Dentsply,Tulsa dental,USA
17	Biodentine	Septodont, Saint Maur, France
18	Bone cement	Palacos bone cement, Hereaus, USA
19	Endosequence root repair material	Brasseler, Savannah, USA
20	MTA carrier	Dentsply,Tulsa dental,USA
21	Non standardised hand plugger	GDC dental, India
22	Nail varnish	Lakme, India
23	Rhodamine B flourescent dye	Sigma-Aldrich corporation, st.louis, MO

S.no	Equipments used	Brandname / Manufacturer details
1	Ultrasonic scaler	Satelec, Acteon, France
2	Micromotor handpiece	NSK pana-max plus, Nakanishi international, Tokyo
3	Dental operating microscope	Ace, Sanma medivisions, India.
4	Radiovisiography	Satelec, Acteon, France
4	Light cure unit	Satelec, Merignac, France
5	Ultrasonic tips	AS6D, Endosuccess, Satelec, Acteon, France
6	Amalgamator	Dental amalgamator, SYG200, NCDS Dental, China
7	Microtome	Leica SP1600 Hard tissue microtome, Germany.
8	Confocal laser scanning microscope	LSM 510 META NLO, Axiovert 200; Carl Zeiss Ltd, Jena, Germany.

Sample Selection

Eighty extracted human maxillary central incisors with straight canals and closed apex were selected for this study (fig 3.A). Teeth with deep root concavities, root caries, incomplete apex and curved canals were excluded. The selected teeth were cleaned with ultrasonic scaler to remove calculus and any remnants of soft tissue and were stored in 0.9% saline solution at 37°C until the preparation. Clinical crowns were sectioned transversely (fig 3.B) using a diamond disc mounted to a slow speed straight handpiece (fig 3.C) under continuous air/water spray to create a standardized root length of 16 mm for all samples (fig 3.D).

Root canal instrumentation

The working length (WL) was established microscopically under 10x magnification by passing an ISO size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) (fig 2.A) in the canal until the tip of the instrument was visible at the apical foramen. The canals were instrumented to the working length up to a size 50 K-file (Dentsply Maillefer, Ballaigues, Switzerland) (fig 3.E). Between every instrument, the canals were irrigated with 5% NaOCl (fig 2.B) followed by saline rinse (fig 2.D). Then final irrigation was done using 17% EDTA (fig 2.F) for 1 minute to remove the smear layer followed by saline. All the canals were then dried using paper points (fig 2.G). The root canals were coated with AH-plus sealer (Densply, USA) (fig 2.H) using a lentulospiral and were obturated with 2% Gutta-percha with size 50 (Diadent Inernational, Canada) (fig 2.I) as master cone followed by accessory cones using cold lateral condensation technique (fig.3.F). The quality of obturations were verified radiographically (Satelec, Acteon, France) (fig 3.G). Coronal opening for each sample was sealed with composite resin (G-aenial, GC Corp, Tokyo, Japan)

which was light cured for 40 seconds. All teeth were subsequently stored in saline at 37°C for one week.

Root end preparation

Roots were resected at 90º to the long axis of the root with a diamond disc (fig 3.I) at 3 mm from the apex (fig 3.H) using a straight handpiece with water coolant (fig 3.J). Retro preparation was made in all the teeth using an ultrasonic retro-preparation diamond tip (AS6D, Satelec, France) (fig 4.A) to a depth of 3mm and 1mm in diameter in a brushing motion with water coolant (fig 4.C). The cavity depth was checked with a graduated periodontal probe to standardize the retro preparation. The preparations were considered complete when no obturation material remained on the cavity walls which were checked using an operating microscope (Ace, Sanma medivisions) under 10x magnification (fig 4.D).

Sample grouping

GROUP	MATERIAL USED	SAMPLE SIZE
Group I	Mineral Trioxide Aggregate	n = 20
Group II	Biodentine	n = 20
Group III	Endosequence root repair material	n = 20
Group IV	Bone cement	n = 20

Group I

Powder and liquid (fig 1.E) were dispensed in the mixing pad in the ratio of 3:1 and the liquid was gradually incorporated into the powder using the Proroot MTA mixing stick for 1 minute to a putty consistency. The mixed material was

dispensed into the cavity using the MTA carrier (fig 1.F) and condensed into the cavity using a non standardised hand plugger (GDC, India) (fig 2.J).

Group II

The liquid was mixed with powder in the capsule (fig 1.B) in an amalgamator (Dental amalgamator, SYG200, China) (fig 1.C) for 30 seconds at the speed of 4000 rpm. The mix was dispensed into the root end cavity using a MTA carrier and condensed into the cavity using a non standardised hand plugger (GDC, India).

Group III

From the preloaded syringe (fig 1.A), the premixed paste was injected directly into the root end cavity and condensed into the cavity using a non standardised hand plugger (GDC, India).

Group IV

The powder and liquid (fig 1.D) were dispensed on a mixing pad and mixed to a consistency when the cement will not stick to unpowdered surgical gloves. The mix was carried using a plastic spatula into the root end cavity and condensed into the cavity using a non standardised hand plugger (GDC, India).

After the placement of retrofilling materials, the samples were coated with two coats of cavity varnish on the external surface of each root to prevent dye penetration through the exposed dentinal tubules except at apical 1mm. All the roots were wrapped in wet pieces of gauze and stored in 100% humidity for 1 week.

Rhodamine B solution was prepared by diluting 2gm of Rhodamine B fluorescent dye (fig 5.A) in 495ml of distilled water in the concentration of 0.2% by weight. The roots were totally immersed in the Rhodamine B solution for 24 hours. Then the staining solution was replaced with saline and the preparations agitated to remove the excess stain. The washing step was repeated twice and the roots were blotted dry.

For preparing the specimen for microtomal sectioning, the coronal ends of the roots were adhered to an acetate sheet with cyanoacrylate and were placed in a 2 ml disposable spectroscopic cuvette (LP Italiana SPA, Milano, Italy). The cuvette was filled with methylmethacrylate (Vertex^{TM} Self curing; Vertex dental BV; Zeist, Netherlands) and pressurized to two bar for 20 minutes to cure exposing the apical end of root vertically upwards (fig 5.B). The preparation was mounted on a CATSI specimen holder (Struers A/S, Ballerup, Denmark) (fig 5.D). One transverse section for each sample was cut at 1mm from the resected root end using the hard tissue microtome (Leica SP 1600 Hard tissue microtome, Germany) (fig 5.C).

Each specimen (fig 5.E) was then examined as to the extent of dye penetration using confocal laser scanning microscope (fig 5.A) and oil immersion objectives with illumination by red laser (543 nm) under 20x magnification. NFT 545 was used as a secondary dichroic mirror. A 560nm long pass filter was used to visualise the Rhodamine B dye. Rhodamine B dye gave a red-orange fluorescence when excited with green light of 543 nm wavelength. Images (fig 7A-H) were viewed using LSM software and the amount of dye penetration was measured in µm using AIM software.

COMPOSITION OF ROOT END FILLING MATERIALS

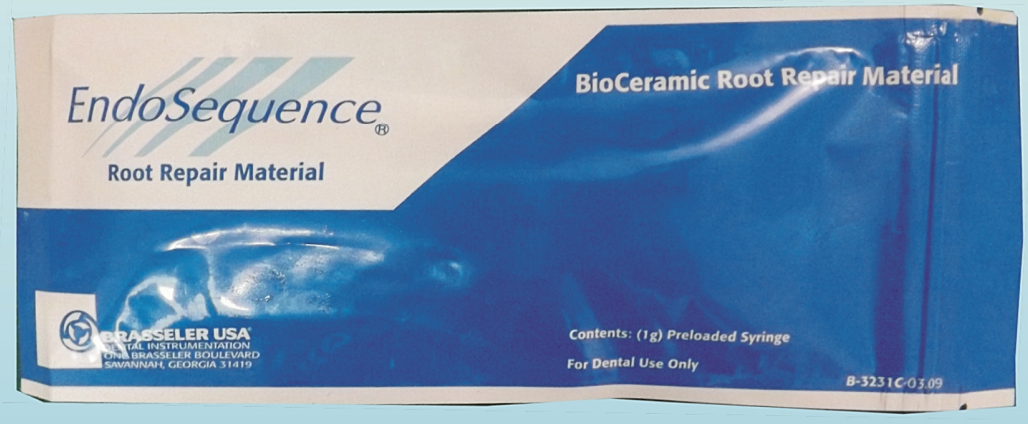
PROROOT MTA	
POWDER	LIQUID
Tricalcium silicate	premeasured unit dose of water
Dicalcium silicate	
Tricalcium aluminate	
Tetracalcium aluminoferrite	
Gypsum	
Free calcium oxide	
Bismuth oxide	

BIODENTINE	
POWDER	LIQUID
Tricalcium silicate	Calcium chloride in aqueous solution
Dicalcium silicate	Admixture of polycarboxylate
Calcium carbonate	
Zirconium dioxide	

ENDOSEQUENCE ROOT REPAIR MATERIAL
Calcium silicate
Zirconium oxide
Tantalum oxide
Calcium phosphate monobasic
Filler agents

BONE CEMENT	
POWDER	LIQUID
Polymethyl methacrylate/copolymer	Methyl methacrylate
Benzoyl peroxide (initiator)	N,N-Dimethyl para-toluidine (accelerators)
Barium sulphate (radiopacifiers)	Hydroquinone (stabiliser)
Zirconia (radiopacifiers)	
Antibiotics (e.g., gentamicin).	

1. ROOT END FILLING MATERIALS



1. A. ENDOSEQUENCE ROOT REPAIR MATERIAL

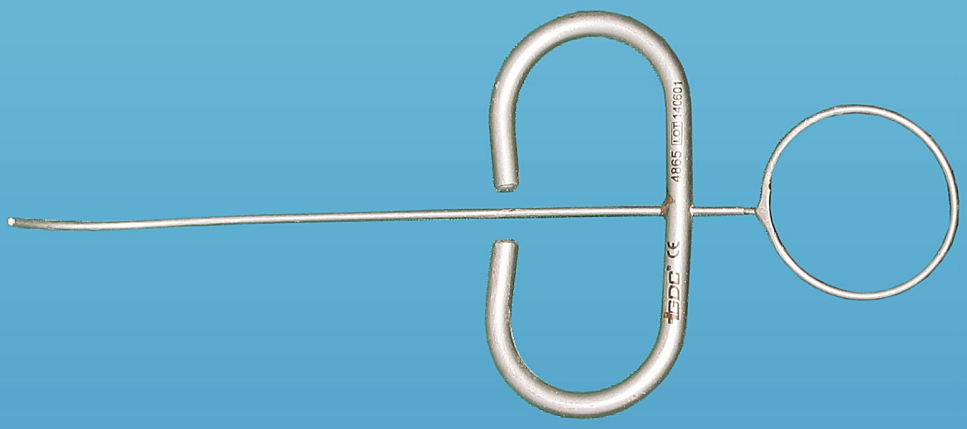
1. B. BIODENTINE

1. C. AMALGAMATOR

1. ROOT END FILLING MATERIALS

1. D. BONE CEMENT

1. E. MINERAL TRIOXIDE AGGREGATE



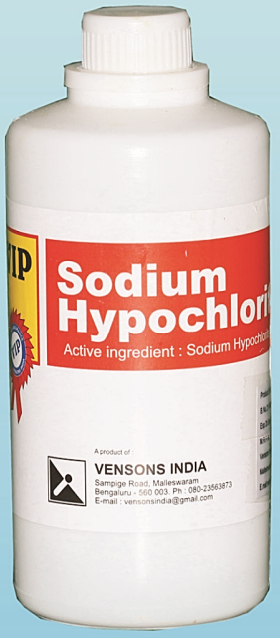
1. F. MTA CARRIER

2. ARMAMENTARIUM FOR ROOT CANAL PREPARATION

2.A. K - FILES

2.B. GATES GLIDDEN DRILLS

2. ARMAMENTARIUM FOR ROOT CANAL PREPARATION



2.C. SODIUM HYPOCHLORITE

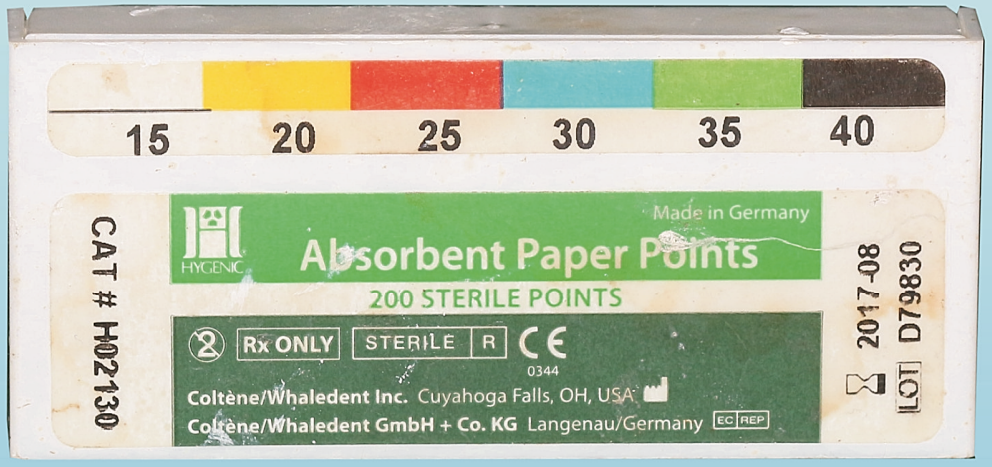
2.D. NORMAL SALINE

2. ARMAMENTARIUM FOR ROOT CANAL PREPARATION

2.E. EDTA LUBRICANT

2.F. EDTA IRRIGANT

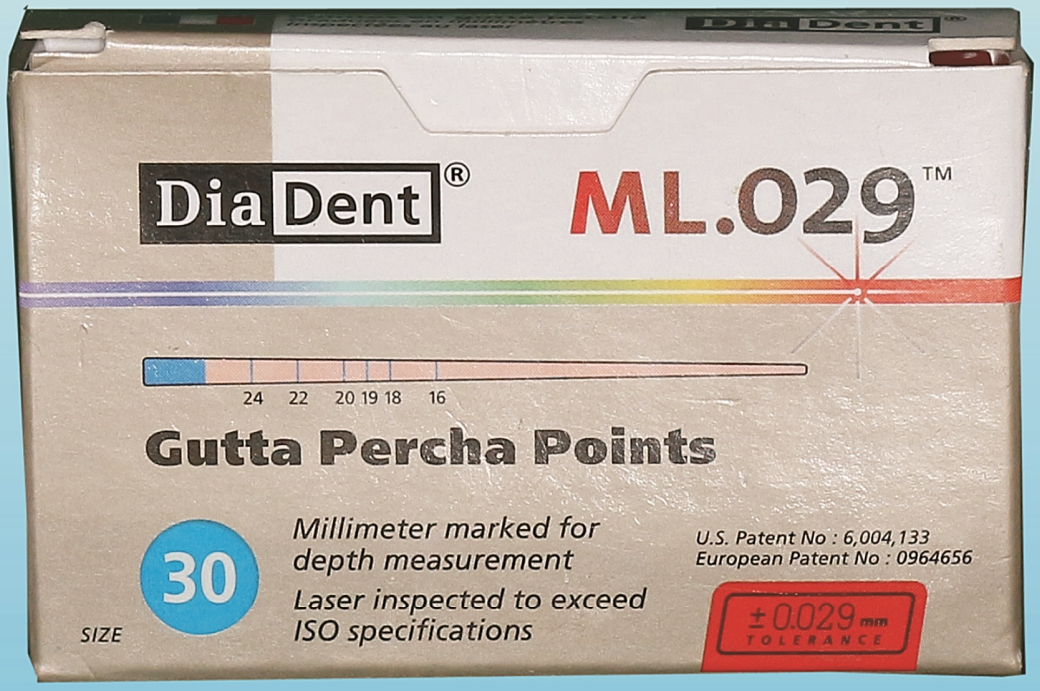
2. ARMAMENTARIUM FOR ROOT CANAL PREPARATION



2.G. PAPER POINTS

2.H. RESIN SEALER

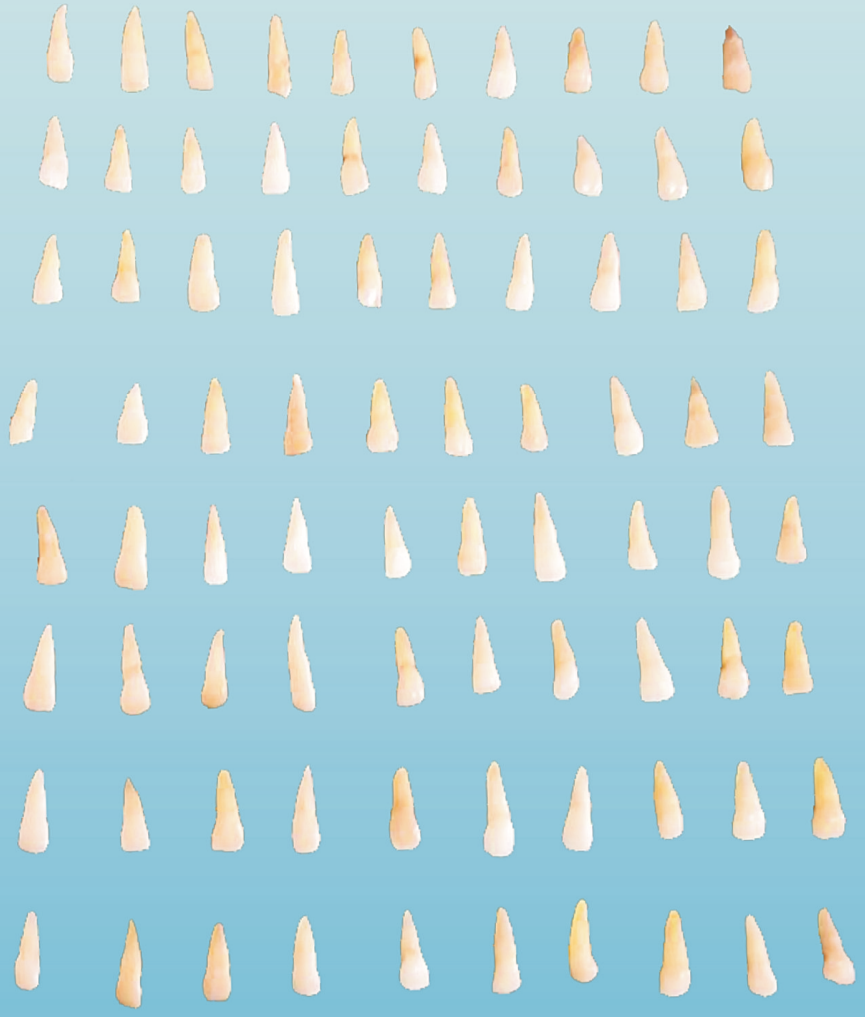
2. ARMAMENTARIUM FOR ROOT CANAL PREPARATION



2.I. GUTTA PERCHA POINTS

2.J. HAND PLUGGER

3. STEPS IN TOOTH PREPARATION



3.A. COLLECTION OF TEETH

3.B. TOOTH MARKED AT 16 mm

3. STEPS IN TOOTH PREPARATION

3.C. DIAMOND DISC MOUNTED ON STRAIGHT HAND PIECES

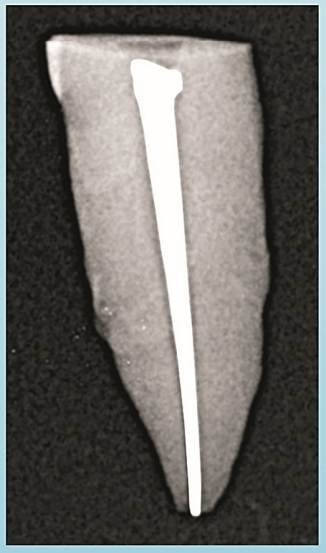
3.D. CORONAL RESECTION

3. STEPS IN TOOTH PREPARATION

3.E. CLEANING AND SHAPING - STEP BACK METHOD

3.F. OBTURATION - LATERAL CONDENSATION

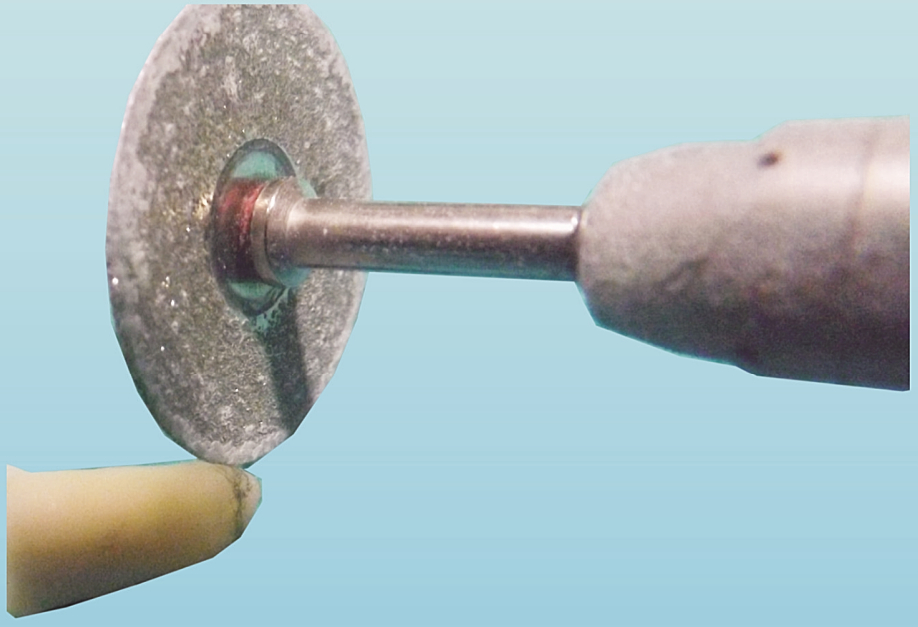
3. STEPS IN TOOTH PREPARATION



3.G.. OBTURATION OF SAMPLE EVALUATED WITH DIGITAL RADIOGRAPH

3.H. OBTURATED SAMPLE MARKED AT 3 MM FROM APEX

3. STEPS IN TOOTH PREPARATION



3.I. APICAL RESECTION USING DIAMOND DISC

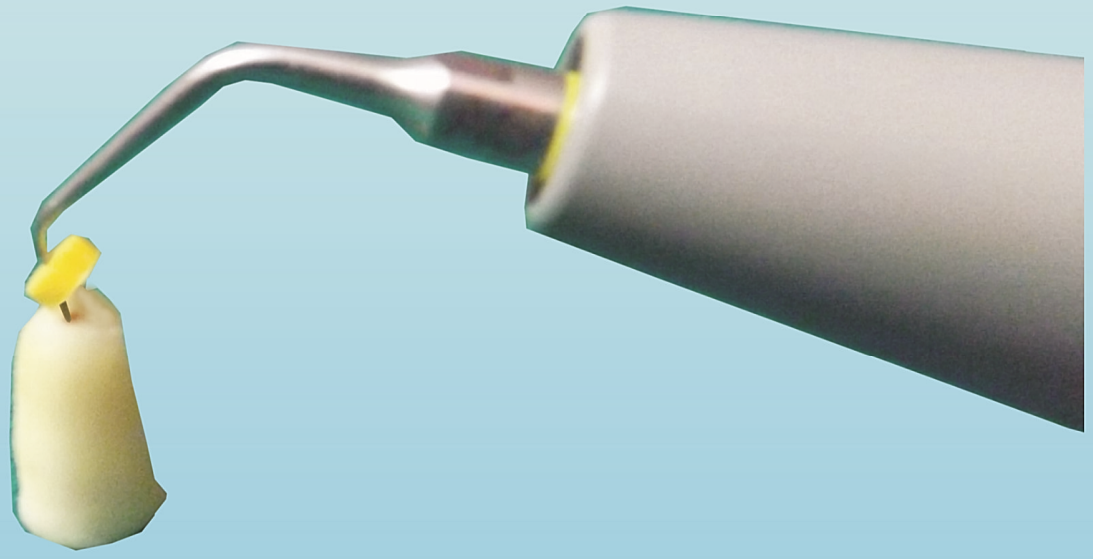
3.J. APICALLY RESECTED ROOTS

4. STEPS IN ROOT END PREPARATION

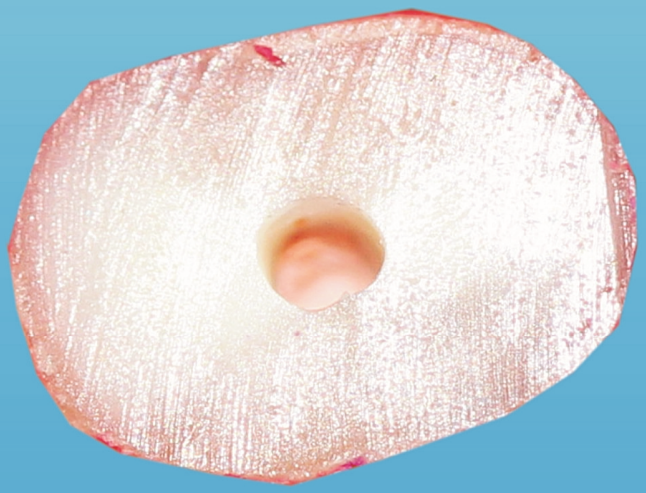
4.A. ULTRASONIC DIAMOND COATED RETROTIP

4.B. RETROTIP MOUNTED ON ULTRASONIC SCALER

4. STEPS IN ROOT END PREPARATION



4.C. ROOT END PREPARATION



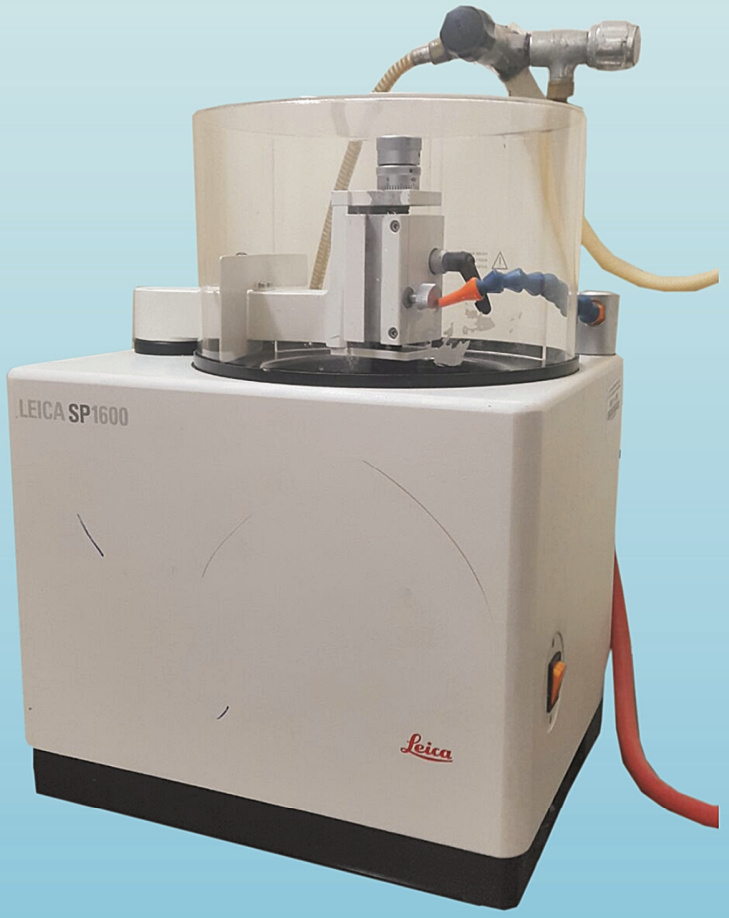
4.D. PREPARED ROOT END CAVITY

5 . TOOTH PREPARATION FOR SECTIONING

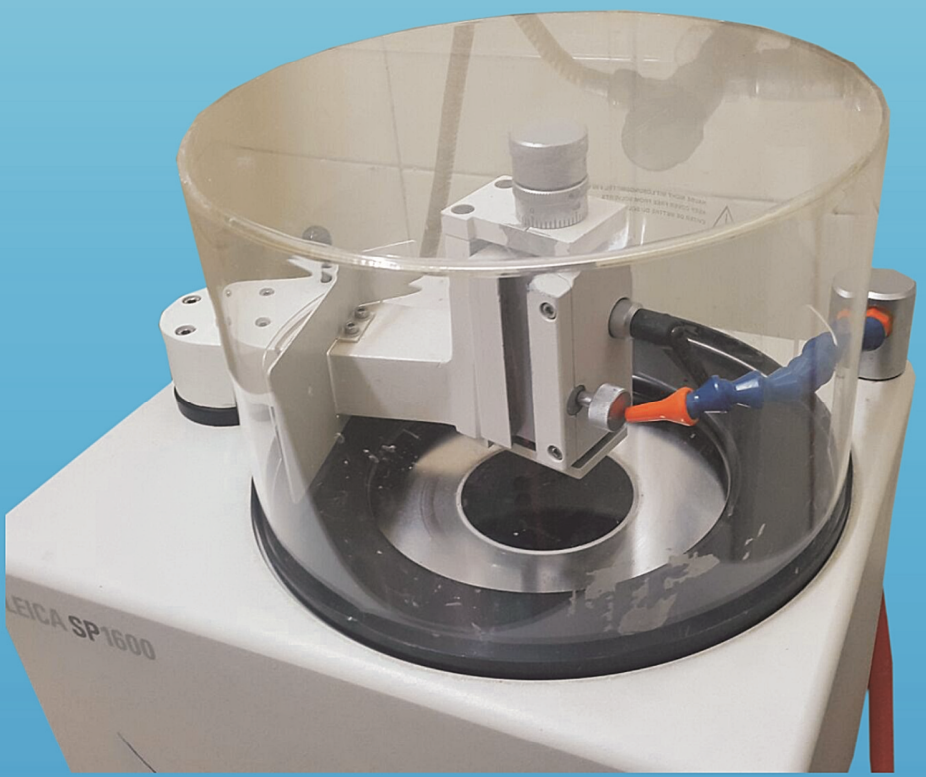
5.A. RHODAMINE B FLOURESCENT DYE

5.B. MOUNTING OF SPECIMEN

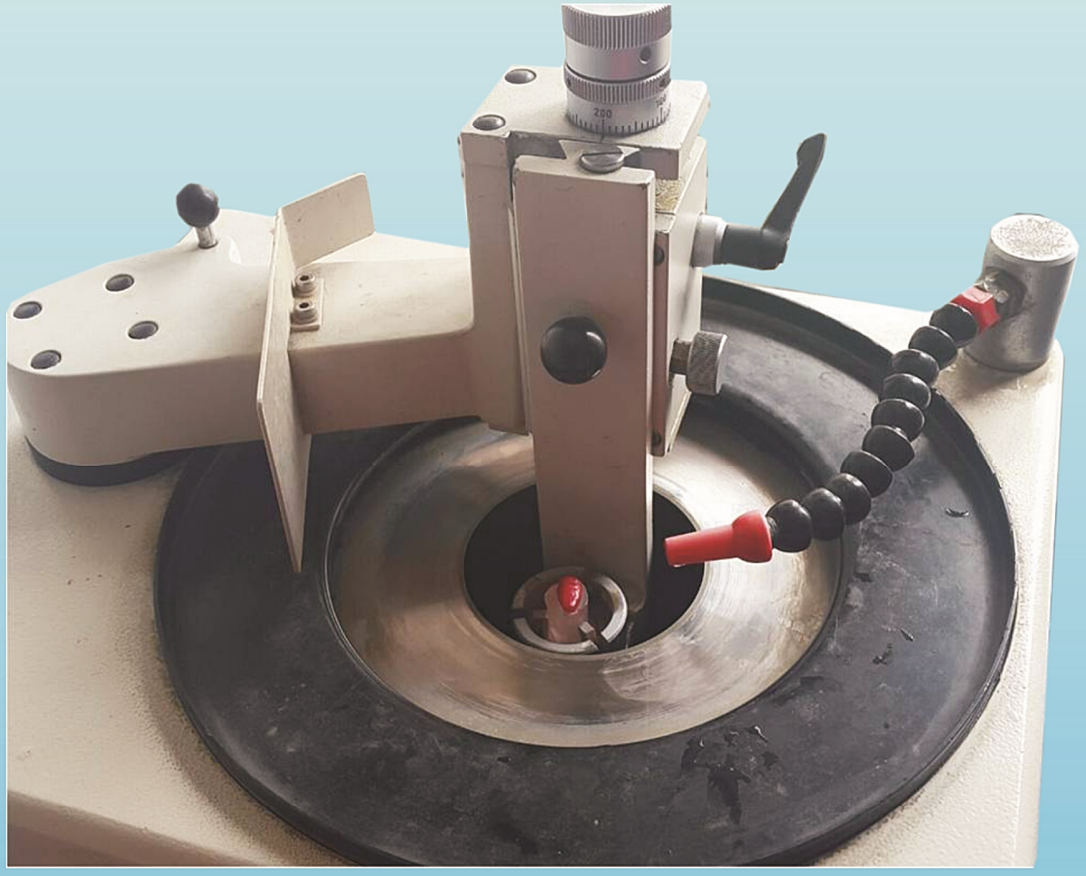
5 . TOOTH PREPARATION FOR SECTIONING



5.C. HARD TISSUE MICROTOME



5 . TOOTH PREPARATION FOR SECTIONING



5.D. MOUNTING OF SPECIMEN IN MICROTOME

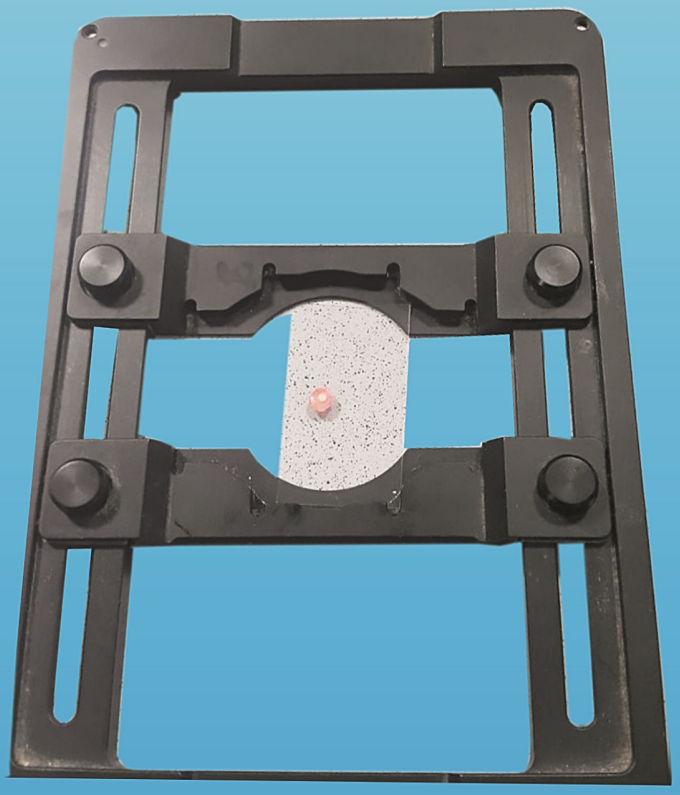
5.E. CROSS SECTION OF SPECIMEN AT APICAL 1 MM

6. CONFOCAL MICROSCOPY

6.A. CONFOCAL LASER SCANNING MICROSCOPE

6. CONFOCAL MICROSCOPY

6.B. OIL IMMERSION MEDIUM



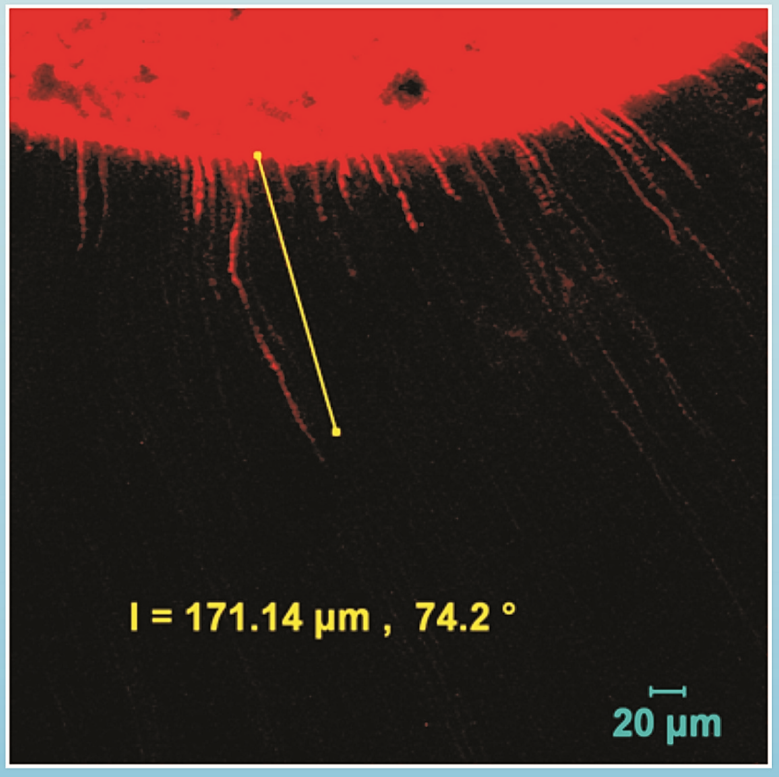
6.C. SPECIMEN MOUNTED ON TRAY

6. CONFOCAL MICROSCOPY

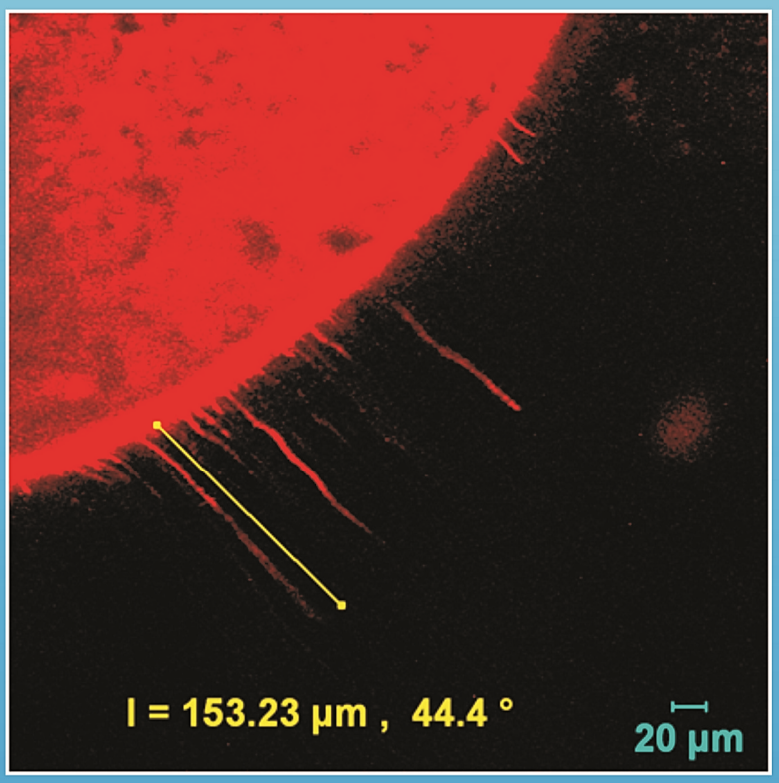
6.D. SPECIMEN VIEWED UNDER MICROSCOPE

6.E. CONFOCAL IMAGE ON LCD SCREEN

7. CONFOCAL IMAGES

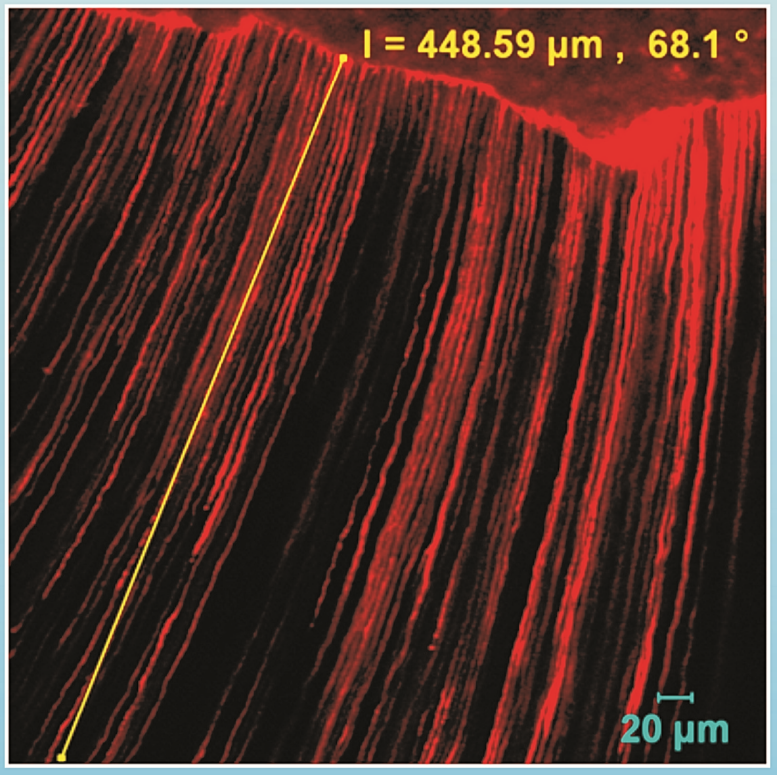


7.A. CLSM IMAGE OF GROUP I - SAMPLE 1

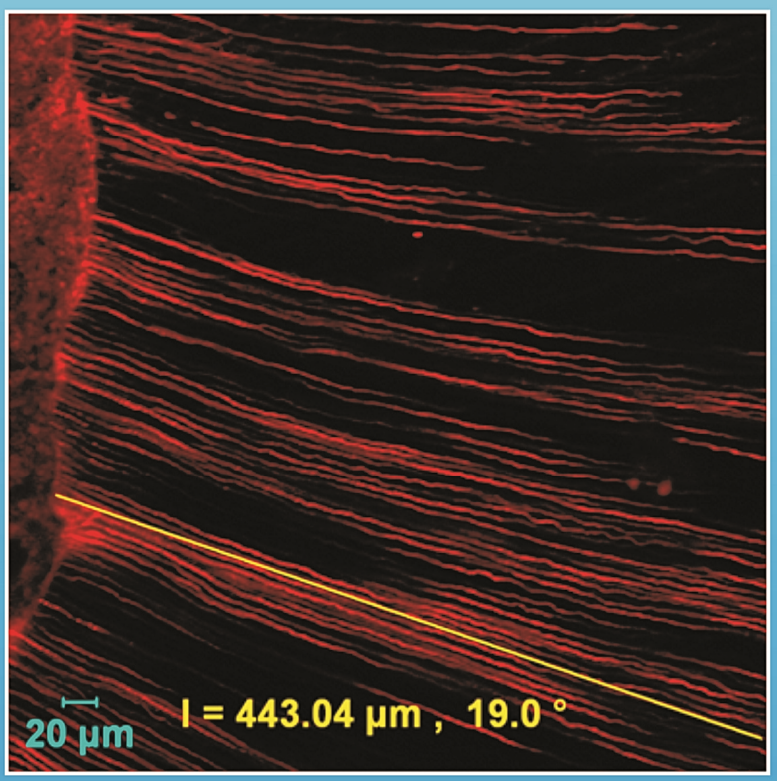


7.B. CLSM IMAGE OF GROUP I - SAMPLE 2

7. CONFOCAL IMAGES

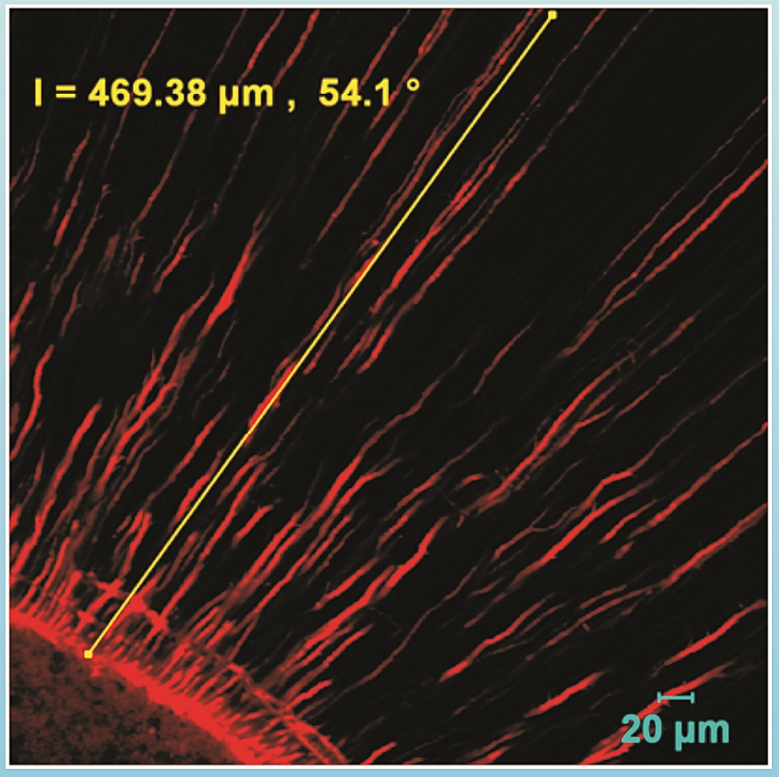


7.C. CLSM IMAGE OF GROUP II - SAMPLE 1

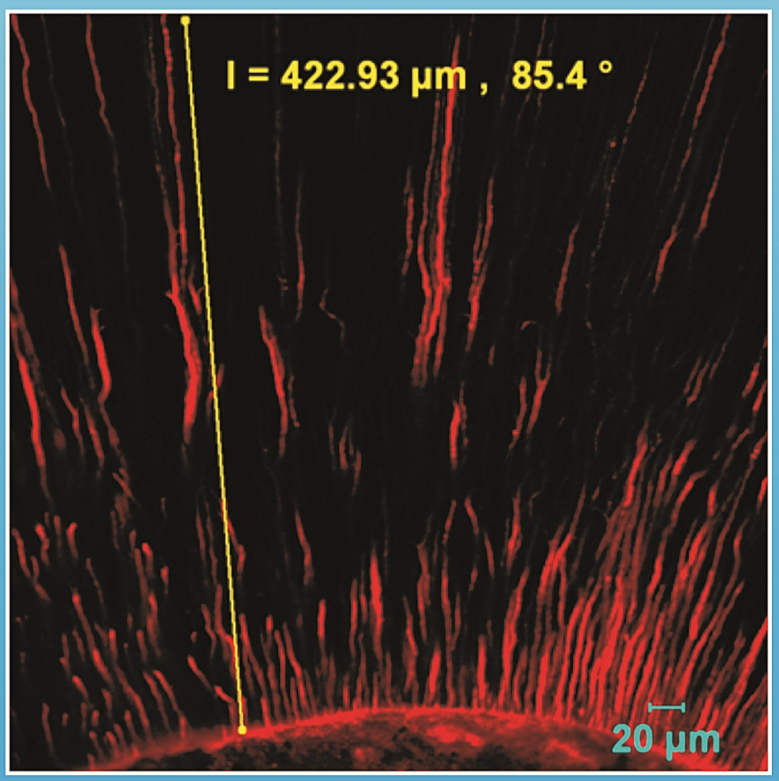


7.D. CLSM IMAGE OF GROUP II - SAMPLE 2

7. CONFOCAL IMAGES

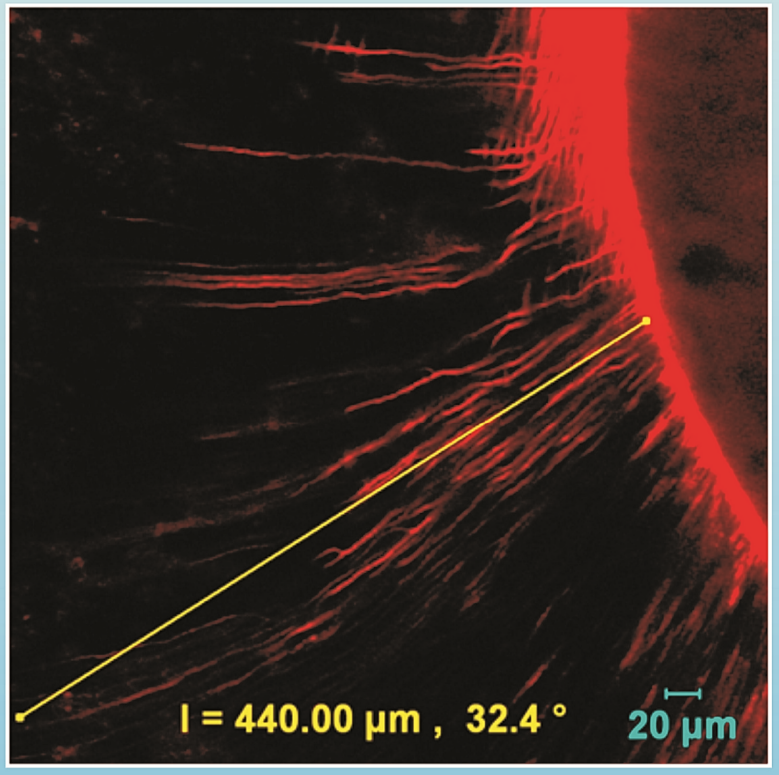


7.E. CLSM IMAGE OF GROUP III - SAMPLE 1

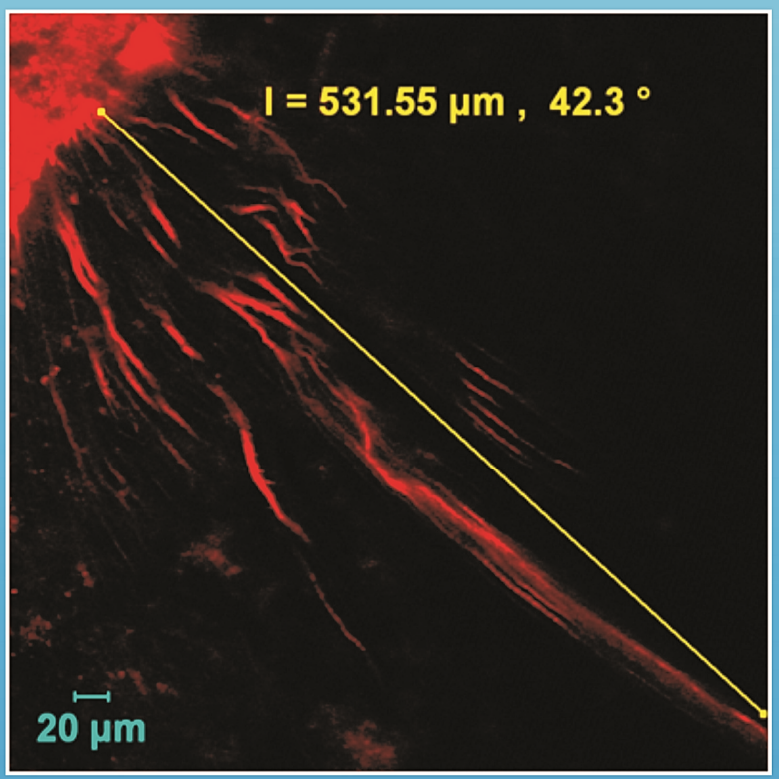


7.F. CLSM IMAGE OF GROUP III - SAMPLE 2

7. CONFOCAL IMAGES



7.G. CLSM IMAGE OF GROUP IV - SAMPLE 1



7.H. CLSM IMAGE OF GROUP IV - SAMPLE 2

RESULTS

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Sciences (SPSS) Software version 17. The data entry was done with Microsoft office excel spread sheet. Both descriptive and analytical statistics were performed. Descriptive statistics include mean and standard deviation (SD) for all the parameters. The mean value for the depth of penetration was calculated separately for the four groups. Analytical statistics includes Analysis of variance (ANOVA) and to find out the significance among the four different groups, a multiple comparison Post hoc using Bonferroni was done. For the entire analysis p value less than 0.005 (Bonferroni correction) was only considered significant. This implicates the probability of committing a type 1 error is of less than 5%.

RESULTS

Table 1 shows the values of maximum and minimum depth of penetration of Rhodamine B dye into the dentinal tubules which corresponds to the microleakage values of the four materials. Among the four groups, the minimum microleakage was shown by MTA group and the maximum microleakage value was shown by Biodentine.

Table 2 shows the descriptive analysis of the four groups which was done using one way ANOVA. The mean value of microleakage for MTA group was 241. 43 μm, Biodentine group was 476.48 μm, ERRM group was 457.30 μm, Bone cement group was 377.79 μm. The F value was 47.59 and p value less than 0.005 was only considered significant. This shows that the differences in mean microleakage among the four groups were statistically significant.

Table 3, 4, 5 and 6 shows the intergroup comparison of microleakage between the four groups. The comparison was done with Post hoc analysis using Bonferroni test. Table 3 shows the comparison of MTA with other 3 groups. The mean difference between MTA and Biodentine was 235.04 μm and p value was statistically significant. The mean difference between MTA and ERRM was 215.87 μm and p value was statistically significant. The mean difference between MTA and Bone cement was 136.36 μm and p value was statistically significant.

Table 4 shows the comparison of Biodentine with other 3 groups. The mean difference between Biodentine and MTA was 235.04 μm and p value was statistically significant. The mean difference between Biodentine and ERRM was 19.17 μm and p value was 1.000 which was not statistically significant. The mean difference between Biodentine and Bone cement was 98.68 μm and p value was statistically significant.

Table 5 shows the comparison of ERRM with other 3 groups. The mean difference between ERRM and MTA was 215.87 μm and p value was statistically significant. The mean difference between ERRM and Biodentine was 19.17 μm and p value was 1.00 which was not statistically significant. The mean difference between ERRM and Bone cement was 79.51 μm and p value was 0.003 which was statistically significant.

Table 6 shows the comparison of Bone cement with other 3 groups. The mean difference between Bone cement and MTA was 136.36 μm and p value was statistically significant. The mean difference between Bone cement and Biodentine was 98.68 μm and p value was statistically significant. The mean difference between Bone cement and ERRM was 79.51 μm and p value was 0.003 which was statistically significant.

Graph 1 shows the mean of microleakage values of the four tested materials. Peak value was shown by Biodentine which shows a microleakage of 476.48 µm which was followed by ERRM which shows a microleakage of 457.30 µm which was followed by Bone cement with a microleakage of 377.79 µm. Least value was shown by MTA which shows a microleakage of 241.43 µm.

ONE WAY ANOVA

	Minimum	Maximum
MTA	102.27	390.49
BIODENTINE	411.25	582.41
ERRM	392.53	539.46
BONE CEMENT	218.38	454.35
Total	102.27	582.41

Table 1. Minimum and Maximum values of depth of penetration

GROUPS	**Sample size (n)**	**Standard Deviation (SD)**	**F Value**	**P value**
I-MTA	20	90.25572	47.598	0.000
II- Biodentine	20	46.94662		
III- ERRM	20	48.52270		
IV- Bone Cement	20	80.41340		

Table 2. Descriptive statistics of depth of penetration in four groups

POST HOC TESTS

Multiple Comparison of One Group Vs Other Groups

MTA Vs Other groups

(I) Group	(J) Group	Mean Difference	Std. Error	Sig.
MTA	BIODENTINE	235.04*	21.89226	0.000***
	ERRM	215.87*	21.89226	0.000***
	BONE CEMENT	136.36*	21.89226	0.000***

Table 3. Comparative analysis of MTA with other groups

BIODENTINE Vs Other Groups

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
BIODENTINE	MTA	235.04*	21.89226	0.000***
	ERRM	19.17	21.89226	1.000NS
	BONE CEMENT	98.68*	21.89226	0.000***

Table 4. Comparative analysis of Biodentine with other groups

POST HOC TESTS

Multiple Comparison of One Group Vs Other Groups

ERRM Vs Other groups

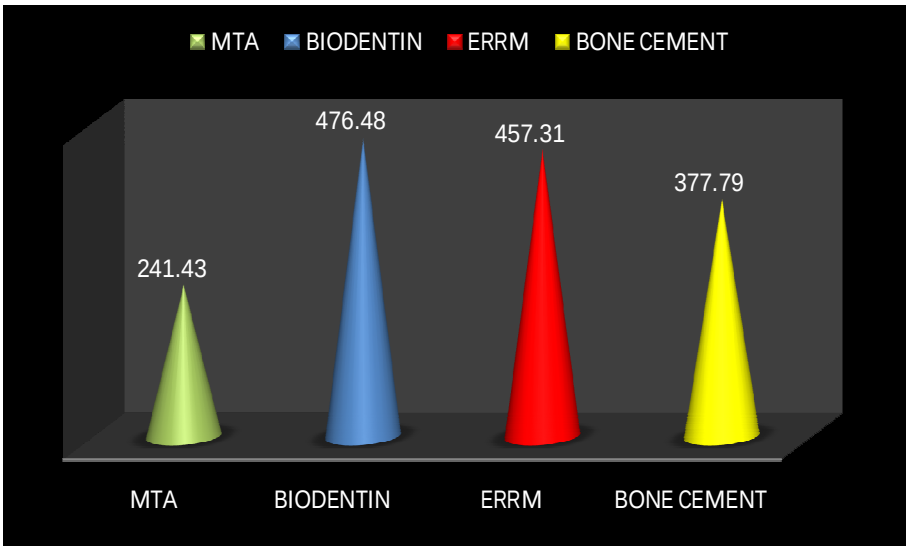
(I) Group	(J) Group	Mean Difference	Std. Error	Sig.
ERRM	MTA	215.87*	21.89226	0.000***
	BIODENTINE	19.17	21.89226	1.000NS
	BONE CEMENT	79.51*	21.89226	0.003***

Table 5. Comparative analysis of ERRM with other groups

BONE CEMENT Vs Other groups

(I) Group	(J) Group	Mean Difference	Std. Error	Sig.
BONE CEMENT	MTA	136.36*	21.89226	0.000***
	BIODENTINE	98.68*	21.89226	0.000***
	ERRM	79.51*	21.89226	0.003***

Table 6. Comparative analysis of Bone cement with other groups



Graph 1. Mean values of Microleakage of four groups

DISCUSSION

The major cause of periapical lesions is an inadequate apical seal which leads to spread of microorganisms and their toxins beyond apex. The removal of the diseased periapical tissues by periradicular curettage eliminates only the effect of the leakage and not the cause. Thus, the elimination of the periradicular lesion alone will likely result in the recurrence of the lesion[1]. Initially, there may be a cessation of symptoms and a radiographical improvement, but often this is transitional. As the initial healing surge plateaus, the slower but persistent pathosis prevails and the case would eventually fail again. For such failed nonsurgical case, nonsurgical retreatment should be attempted first, provided that the benefits of this retreatment outweigh the surgical retreatment. Apical surgery entails not just the removal of the diseased tissue or the root tip, but most importantly resealing of the root canal system[2].

Altonen and Mattila[62] reported that teeth with root-end fillings had greater healing success than those that were not filled, provided that nonsurgical fillings were done. Lustman and Rahbaran[63] also observed better success with either amalgam or Super EBA root-end filling materials than in the non-filled groups. The materials and surgical techniques used in these studies were of the traditional methods. Regardless, it was clear from these studies that filling the root ends with retrograde filling materials results in greater healing success.

As none of the root filling materials available today are capable of forming a hermetic seal blocking all the portals of entry between the intraradicular and extraradicular systems, there comes the need of a root end filling material which would provide an optimal seal. A retrograde root filling is placed to establish an apical seal to prevent the passage of microorganisms or their products into periapical

tissues. Apical seal is the single and most important factor in achieving success in surgical endodontics[2]. Though a plethora of root end filling materials are available, it is the sealing ability of a material which makes an ideal root end filling material which prevents leakage of irritants and thereby aids in complete healing. So this study was aimed at evaluating the sealing ability of four new root end filling materials which is of clinical relevance in choosing a root end filling material.

Eighty extracted human central incisors were chosen for this study. The decision to select roots with straight canals were made because curved canals are associated with a more complicated curvature determination and distribution process and with an increased risk of intracanal procedural accidents such as, zipping, ledging and root perforation[64]. Moreover, curvatures if present cannot be standardised as it differs among each tooth. So straight canals offer a more standardized method for evaluation of apical leakage. So this study was aimed to evaluate the sealing ability in teeth with straight canals.

Beling *et al*[65] reported that there was no significant difference between prepared and unprepared teeth. On the other hand, Gulabivala *et al*[66] found a statistically significant difference between prepared and unprepared teeth in terms of crack formation. In this study, the root canals were prepared and obturated with gutta-percha to mimic clinical conditions.

Obturation of root canals were performed using lateral condensation technique, which is of easy execution and efficacy as proved by Bonetti *et al*[67]. AH Plus was proved to be the endodontic sealer showing the smallest apical leakage mean values in teeth filled with gutta-percha points, exhibited the best results when compared with the other groups as proved in study by Oliveira *et al*[68]. So AH Plus

sealer has been used to coat the canal walls and gutta-percha used for obturation in this study.

Gilheany *et al*[69] proved that at least 2 mm of root apex be removed to minimize bacterial leakage from the canals. The anatomical study of the root apex shows that at least 3 mm of the root-end must be removed to reduce 98% of the apical ramifications and 93% of the lateral canals. A root-end amputation of less than 3 mm does, most likely, not remove all of the lateral canals and apical ramifications, therefore, posing a risk of reinfection and eventual failure[70].

The aim of the root-end preparation is to remove the intracanal filling material and irritants and to create a cavity that can be properly filled. The ideal root-end preparation can be defined as a class 1 cavity at least 3 mm into root dentine, with walls parallel to and coincident with the anatomic outline of the root canal space[71].

The vast majority of root-end cavity preparation-related failures are attributed to irregular cavity configuration in the vestibulo-oral dimension, inability to prepare deep and parallel cavity walls and creation of cracks during cavity preparation[72]. In addition, a well-adapted filling material in a properly prepared root-end cavity is a vital factor in the success of surgical retreatment procedure[73] .

The resection of the root should be perpendicular to the long axis of root devoid of bevels. A 45º buccolingual bevel is proved to facilitate the material insertion, and can be indicated when conventional handpiece is used for cavity preparation. However, it could increase the apical leakage because the number of dentinal tubules exposed are more thereby the increasing the permeability[36]. There is no biological justification for a steep bevel angle. It was strictly for the convenience of the surgeons for identifying the root apex and for the subsequent apical preparation.

Bevelling also causes significant damage to the tissue structures such as buccal bone and root which are intended to be saved. By diagonal resection, the result of steep bevelling, the buccal bone is removed along with a large area of the root causing, in effect, a large osteotomy. Furthermore, bevelling frequently misses the lingually positioned apex, causes elongation of the canal and reduction of the root diameter, thereby weakening it[46]. So the root should be resected as perpendicular to the long axis of the root as possible which has been done in this study with the aid of diamond disc.

Improvement in sonic and ultrasonic (US) retrotips have been a great leap in root end treatment. US retrotips is advantageous over traditional apical surgery with high speed handpieces and burs as it creates smaller, better centered, and better shaped root end cavities thereby reducing the risk of perforation[74]. Also, these devices can follow the long axis of the tooth, and apical cavities can be prepared easily and safely. In addition, the cutting bevel on the resected root end can be made perpendicular to the long axis of the root canal, decreasing the number of exposed dentinal tubules and subsequently decreasing the apical leakage which was ideal for this study[75].

The use of ultrasound-activated tips for root-end cavity preparation improves the outcome of the procedure since less removal of bone tissue is required to gain proper access to the apical region. In addition, ultrasonically prepared root-end cavities can be more conservative than bur-prepared cavities, involving both the canal and the isthmus, allowing better adaptation of the retrofilling material and consequently improving the apical seal[76]. Wuchenich *et al*[77] compared the root-end cavities prepared with conventional handpieces or ultrasonic tips in cadavers in a SEM study. They found that ultrasonics tips made cleaner and deeper root-end cavity

preparations, aiding retention of the root-end filling material and disinfection by removing infected dentin.

Aqrabawi[20] stated that if root end filling materials were able to prevent the leakage of small particles such as dye, they would possibly prevent the penetration of bacteria and their byproducts. So this study was designed to apply Rhodamine B dye to evaluate the sealing ability of root end filling materials by measuring the penetration of Rhodamine B dye into dentinal tubules which measures the extent of microleakage using confocal microscope[78]. As Rhodamine B which is a water-soluble fluorescent dye is easily detectable, even in a low concentration, can move freely along the interface, of low toxicity, stable in an aqueous environment, stable in varying pH and non-destructive to the substrate or material in contact it was chosen for this study.

Transverse sectioning has been chosen for the specimen preparation as it was proved by Torabinejad *et al*[15] that transverse sections allow better visualisation of the material-dentin interface throughout the circumference. Hard tissue microtome has been used to perform the transverse sectioning of tooth as it enables specimens of a useful thickness and resolution to be prepared while leaving the enamel and dentine in their natural state. To reduce the risk of section damage, cutting and precision grinding of the tooth specimens have been done with the teeth embedded in artificial resin so that they have been stabilised against stress[79].

Among the wide range of microscopes available, CLSM has been selected for this study as it definitely proves to be advantageous over the commonly used light microscope and scanning electron microscope. Light microscope does not have enough depth of focus or resolution and scanning electron microscope can create

artifacts as it needs complex specimen preparation method and drying of specimens. These disadvantages have been elliminated with the selection of confocal microscope which is a fast, precise and non-tactile method and does not need drying of samples leaving the sample artifact free and it gives a high resolution three dimensional image[80].

The results obtained in this study showed that the sealing ability of MTA was found to be superior compared to the other three retrofilling materials used in this study. The potent sealing ability of MTA was probably due to its hydrophilic nature, setting expansion and mineralisation potential. Studies by Chen *et al*[58] and Shahi *et al*[81] have proved that the sealing ability of MTA was attributed to its ability of inducing mineralisation which has been proved by messenger RNA expression of mineralised tissue proteins of cementoblasts and bone cells which helps in cemental and osseous repair and regeneration. These findings have been further proved by Yasuda *et al*[82] who have evaluated the mineralisation ability of MTA in rat dental pulp cells and found that MTA stimulated about 60% mineralisation and regulated Bone morphogenic proteins-2 mRNA expression.

Camilleri *et al*[83] studied the hydration reaction of MTA and showed that on hydration, MTA becomes a colloidal gel which undergoes solidification to form a strong impermeable barrier. The release of calcium ions from MTA which contributes to the production of hydroxyapatite has been proved by Duarte *et al*[84], Santos *et al*[85] and Sarkar *et al*[86]. The hydroxyapatite formation was further accelerated by the high pH of MTA on setting which changes from 10.2 to 12.5 in 3 hours creating an alkaline environment favouring hydroxyapatite formation and a decrease in calcium phosphate solubility.

The mechanism of hydroxyapatite formation was evaluated by Han *et al*[87] who identified precipitates on the surface of MTA as calcium hydroxide and calcium carbonate when MTA comes in contact with distilled water and precipitates of amorphous calcium phosphate when comes in contact with phosphate buffered saline, which would eventually transform to apatite crystals. Reyes Carmona *et al*[88] using x-ray diffraction and SEM methods found that MTA triggers carbonated apatite precipitation which forms an interfacial layer with tag like structures which promotes a controlled mineral nucleation on dentin. This mechanism further makes the cement retentive to dentin through a micromechanical bonding system which shows greater resistance to displacement which obviously attributes to the sealing ability[89].

Xavier CB *et al*[68] stated that MTA powder consists of fine hydrophilic particles that absorb water during hydration which has occurred on being cured in a moist environment. Therefore, the material expands during solidification, which must have played a role in its superior adaptation to cavity margins. This was further supported by Torabinejad *et al*[19] who concluded that hydration of the powder might have resulted in formation of the colloidal gel that gets solidified to a hard structure in less than 4 hours. Camilleri[83] studied the structure of crystals formed during hydration of MTA and found them as long needle like structures which cause the set material to expand and this expansion contributes to the sealing ability of MTA.

The sealing ability of MTA has been proved to be superior compared to many other materials in comparative studies conducted by various authors. Torabinejad *et al*[15] using Rhodamine B dye found that the marginal leakage was less for MTA compared to amalgam and Super EBA when viewed under confocal microscopy

which is in accordance with our study. During a one year period, using a fluid transport model, Wu *et al*[90] found glass ionomer cements and MTA leaked less than amalgam and EBA cement. A bacterial leakage study by Howard Fogel *et al*[91] to determine the time needed for *Serratia marcescens* to penetrate a 3 mm thickness of amalgam, IRM, EBA cement, and MTA when these materials were used as root end filling materials, showed that MTA was the most effective root end filling material against penetration of *S. marcescens*[92]. The results of this study were in accordance with all these previous studies.

Sealing ability of Bone cement was found to be inferior to MTA but superior to ERRM and Biodentine. PMMA Palacos Bone cement was found to have an excellent adaptation to the cavity margins which was in accordance with the results of the dye diffusion study performed by High and Russell[18], which proved a satisfactory marginal seal by the use of Bone cements (CMW and Palacos with gentamicin) as root-end filling materials. In addition, Holt and Dumsha[20] showed that Simplex P Bone cement provided an acceptable retrofill seal in their leakage study. Though there is polymerization shrinkage associated with acrylics, it provides proper adaptation to the dentinal wall as explained by the results obtained by Charnley *et al*[93], who used a fluid displacement model and observed that the volume of cement increases to a maximum during polymerization before shrinking thereby compensating the polymerisation shrinkage.

One of the problems of methylmethacrylate Bone cement is the exothermic reaction of curing, and as a result, high temperature might be generated during its polymerization[94]. But study by Blinc *et al*[95] suggested a negligible thermally induced effect of Palacos Bone cement because of the small mass of the

cement. In addition, Bone cement tolerates a moist environment very well. Another problem is the component of Bone cement that might be toxic, especially the residual methyl methacrylate monomer. However, many studies have investigated the effect of free monomer on tissue, and they indicated little toxicity[96]. Other studies suggested long-term compatibility of bone with Bone cement, which allowed excellent interlocking of the cement with the soft and hard tissue of bone without bone necrosis. So the excellent sealing ability and good marginal adaptation of Bone cement has been attributed to the increase in volume of cement during polymerisation[97]. But the drawback of Bone cement is the lack of bioactivity which is the desirable property of tricalcium silicate based materials.

The results of this study showed that the sealing ability of EndoSequence Root Repair material was superior to Biodentine but inferior to MTA and Bone cement. ERRM specifically has been created as a white coloured premixed cement for both permanent root canal repairs and apico retrofillings. As a true bioceramic cement, the advantages of this new repair material are its high pH (pH >12.5), high resistance to washout, absence of shrinkage during setting, good biocompatibility and excellent physical properties[98]. In fact, it has a compressive strength of 50-70 MPa, which is similar to that of ProRoot MTA. However, a significant upgrade with this material is its particle size, which allows the premixed material to be extruded through a syringe rather than inconsistent mixing by hand and then placement with a hand instrument. The calcium silicate present in ERRM paste gets hydrated to produce a calcium silicate hydrate gel and calcium hydroxide. The calcium hydroxide reacts with the phosphate ions to precipitate hydroxyapatite and water. The water continues to react with the calcium silicates to precipitate additional gel-like calcium

silicate hydrate and the reaction progresses precipitating more hydroxyapatite crystals which indicates its bioactivity[99].

An *in vitro* study by Ian Chen *et al*[50] has observed that in sections of roots filled with both MTA and ERRM, mineralised tissue covering the entire root end surface was observed which was continuous with cementum on the lateral root surface. Thus it has been stated that the sealing ability of ERRM has been attributed to its mineralised tissue inductive and conductive property. Walsh *et al*[100]observed the advantage of hydroxyapatite formation and ultimately a bond between dentin and ERRM contributing to its sealing ability. The sealing ability of this material also depends on the particle size. This bioceramic material is produced with nanosphere particles which facilitates the penetration into dentinal tubules and interacts with moisture present in dentin, creating mechanical bond which also attributes to the sealing ability of ERRM[101].

The bioactivity of ERRM has been proved in study by Shokouhinejad[102] who observed the precipitation of apatite crystalline structures at the material-dentin interface. Hirschberg[48] compared the sealing ability of MTA and ERRM using bacterial leakage study and proved that ERRM leaked significantly more than MTA which is in agreement with the results of this study. Shokouhinejad[98] conducted another *in vitro* study to compare the marginal adaptation of MTA and ERRM paste and observed that ERRM paste leaked more than MTA and this leakage was due to the low viscosity of the material which made it difficult to be compacted properly within the root end cavity which is in accordance with this study.

According to the results obtained in this study, the sealing ability of Biodentine is the least when compared to the other three materials. The setting

reaction of Biodentine which is also a tricalcium silicate based material is more or less the same as that of MTA in creating an alkaline environment and the release of calcium ions[103-107]. The difference between MTA and Biodentine lies in the addition of calcium chloride to the liquid of Biodentine which serves as an accelerator for the setting reaction. Bortuluzzi *et al*[108] evaluated the action of calcium chloride on sealing ability of two types of MTA using dye leakage evaluation method using Rhodamine B dye. They found that the cements added with calcium chloride leaked significantly less.

The Biodentine liquid included a chloride-containing phase and the Biodentine powder had inclusions of calcium carbonate. Calcium carbonate is added by the manufacturer to act as a nucleation site[109]. The calcium carbonate particles were large compared to the cement particles. They also exhibited hydration products around their circumference. Though it does not react with water to form reaction by-product, it allows the formation of reaction rims around it, thus enhancing the hydration and producing a denser microstructure. The presence of a carbonate phase was verified by X-Ray Diffraction and FT-IR analysis[110]. The presence of hydroxyapatite on calcium silicate-based cements has been demonstrated previously in many studies[111,112]. This hydroxyapatite was present on the surface of Biodentinee which implicates its bioactivity potential like other calcium silicate based cements.

Koubi *et al*[113] performed a study on microleakage of calcium silicate based materials and observed a slight expansion in the set Biodentine which too might have attributed to the sealing ability. They also observed the formation of apatite deposits at the interface between Biodentine and dentin walls. They also noted the small size and texture of the gel formed during the setting of calcium silicate cements which

facilitates better spread of the material over dentin leading to better adaptation of Biodentine.

An *in vitro* study by Camilleri *et al*[114]proved that the sealing ability of Biodentine is due to its ability to form surface apatite crystals when comes in contact with tissue fluids. Han and Okiji[115] evaluated the uptake of calcium and silicon by adjacent root canal dentine in the presence of PBS and found the presence of tag like structures in MTA and Biodentine indicating their bioactivity. Sorrentino *et al*[83] evaluated the hydration reaction of Biodentine and found that there was an increase in calcium carbonate content and mineral tag formation leading to the formation of a hybrid zone at the interfacial dentin. Tran *et al*[116] conducted a comparative study between MTA and Biodentine and observed that the thickness of the interfacial hybrid layer in Biodentine group was lower than the MTA group which may the reason behind the poor sealing ability of Biodentine when compared to MTA as proved in this study.

The limitation of this study was the time factor as this study has evaluated the sealing ability of the materials in a short term period so the changes in behaviour of materials with time is lacking. The setting reactions and the reaction byproducts may vary as the cement ages. So in the future, long term studies for evaluation of this parameter is mandatory.

CONCLUSION

Within the limitations of the present study, it can be concluded that

1. All the four materials used in this study exhibited good sealing ability as root end filling materials in ultrasonically prepared root ends under Confocal Laser scanning microscope.
2. MTA showed better sealing ability with less penetration into the dentinal tubules when compared to Biodentine, ERRM and Bone cement.
3. Sealing ability of Bone cement was superior to ERRM and Biodentine but inferior to MTA.
4. Sealing ability of ERRM was superior to Biodentine with no statistical significant difference but inferior to MTA and Bone cement.
5. Biodentine, ERRM and Bone cement also showed promising sealing ability hence they can be considered as alternatives to MTA as root end filling materials.

SUMMARY

This *in vitro* study was done to evaluate the sealing ability of MTA, Biodentin, ERRM and Bone cement when used as root end filling materials in ultrasonically prepared root ends using confocal laser scanning microscope.

For the study, 80 maxillary central incisors with straight canals have been selected. They were cleaned of debris and coronal resection was done using diamond disc to standardize the teeth to a uniform length of 16 mm. After determining the working length the samples were instrumented, cleaned and shaped to working length with K-files using step back technique. Irrigation was done at subsequent intervals using 5.2% sodium hypochlorite and saline. EDTA was used as a lubricant and EDTA irrigant was used as a final rinse to remove the smear layer followed by saline. The canals were dried using paper points. The canals were coated with resin sealer and obturated with 2% gutta-percha points using lateral condensation technique. Obturation was verified radiographically and the roots were coronally sealed using composite resin and light cured. All the samples were subsequently stored in saline for one week. Then the teeth were marked at 3mm from apex and apical resection was done using diamond disc mounted in a straight hand piece at 90^{o} to the long axis of the root.

Retrograde cavities were prepared in the samples using ultrasonic diamond coated retro tip to create cavities of uniform depth and width of 3mm and 1 mm respectively. The cavities were checked for residual filling materials under microscope. The teeth were then divided into four groups- MTA, Biodentin, Bone cement and ERRM. The four materials were mixed according to manufacturer's instructions. For group 1 (MTA), the powder and liquid were dispensed in a paper pad and mixed in 3:1 ratio and filled into the cavity using MTA carrier and condensed

using non standardized hand plugger. For group 2 (Biodentin), the liquid was added to the powder in the capsule and mixed in an amalgamator. The mix was filled in the cavity using MTA carrier and condensed using non standardized hand plugger. For group 3 (ERRM), the premixed paste was dispensed straight into the cavity and condensed using non standardized hand plugger. For group 4 (Bone cement), the powder and liquid were dispensed in a mixing pad and mixed till it becomes non sticky and carried into the cavity using plastic spatula and condensed into the cavity using non standardized hand plugger.

After completion of retrograde obturation the teeth were coated with nail varnish except at apical 1mm and wrapped in wet gauze pieces and kept in 100% humidity. After a week the samples were taken and kept immersed in 0.2% Rhodamine B dye solution for 24 hours. Then they were rinsed in saline and blotted dry.

For sectioning under microtome, the samples were stabilized in a methyl methacrylate resin mould with the coronal end immersed in the mould. Then they were mounted on the specimen holder and transverse sections were made at 1mm from apex. For examining under confocal microscope the sections were stabilized on a tray using an oil immersion medium. The samples were viewed with illumination by red laser (543 nm) under 20x magnification. Rhodamine B dye gave a red-orange fluorescence when excited with green light of 543 nm wavelength. Images were viewed under LSM software and the amount of dye penetration was measured in µm using the AIM software.

The depth of dye penetration indicated the value of microleakage of the materials. So the sealing ability of a material was found to be inversely proportional

to the depth of penetration i.e., lower the value of microleakage, better the sealing ability.

Data were analyzed using SPSS Software version 17. The data entry was done with Microsoft office excel spread sheet. Descriptive statistics include mean and SD for all the parameters. Analytical statistics includes one way ANOVA and the mean value for the depth of penetration was calculated separately for the four groups.

The microleakage of MTA group ranged from 102.27 μm to 390.49 μm, Biodentin group ranged from 411.25 μm to 582.41 μm, ERRM group ranged from 392.53 μm to 539.46 μm, Bone cement group ranged from 218.38 μm to 454.35 μm. The mean value of microleakage for MTA group was 241.43 μm, Biodentin group; 476.48 μm, ERRM group; 457.31 μm, Bone cement group; 377.79 μm. The least value of microleakage was shown by MTA group and highest value of microleakage was shown by Biodentin group.

To find out the significance among the four different groups, a multiple comparison Post hoc using Bonferroni was done. For the entire analysis p value less than 0.005 (Bonferroni correction) was only considered significant. There was a statistically significant difference in microleakage between MTA and the other three groups. But there was no statistically significant difference between Biodentin and ERRM group.

Within the limitations of the present study, it can be concluded that all the four materials used in this study exhibited good sealing ability as root end filling materials in ultrasonically prepared root ends under CLSM. MTA showed better sealing ability with less penetration into the dentinal tubules when compared to Biodentin, ERRM and Bone cement. Biodentin, ERRM and Bone cement also showed

promising sealing ability hence they can be considered as alternatives to MTA as root end filling materials.

Though this study has evaluated the sealing ability of four root end filling materials it was of short term. Further studies on evaluation of a material's sealing ability over a longer duration is very essential. As time has an influence on the sealing ability of various materials, long term studies which predict not only the immediate effect of the setting reaction of the materials but also the capability of the materials to maintain the apical seal created is mandatory to reveal the success of a material as root end filling material.

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ANNEXURES

Estd : 1987

INSTITUTIONAL ETHICS COMMITTEE AND REVIEW BOARD

Rajas Dental College & Hospital

Thirurajapuram, Kavalkinaru,Jn – 627 105, Tirunvelveli District.

DCI Recognition No : DE-3(44)-93/2246
Dated 09-11-1993

Affiliated to
The Tamil Nadu Dr. M.G.R. Medical University, Chennai

This ethical committee has undergone the research protocol submitted by **Dr.G.Priya Johnson**, Post Graduate Student, **Dept of Conservative dentistry & Endodonics** under the title "Comparative evaluation of sealing ability of Mineral Trioxide Aggregate, Biodentine, Endosequence root repair material and Bone cement as retrograde filling materials in ultrasonically prepared root ends using confocal laser scanning microscope– an in vitro study" under the guidance of **Dr. R. Jonathan Emilsam** for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfills the specific requirements and the committee authorizes the proposal.

I.E.C. & R.B. RAJAS DENTAL COLLEGE & HOSPITAL ETHICAL REVIEW BOARD

Dr. I. PACKIARAJ MDS
CHAIR PERSON
Ethical Committee

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